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MELVILLE T. COOK, Editor.

The Sphaerodactyls of Porto Rico, Culebra and Mona Islands, By *Chapman Grant.*

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Observations on the Bean Lace Bug in Porto Rico, By *Mortimer D. Leonard* and *Alfred S. Mills.*

The Haitian Coffee Tree Cricket, By *Charles H. Arndt* and *Herbert L. Dozier.*

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THE SPHAERODACTYLS OF PORTO RICO, CULEBRA AND MONA ISLANDS

CHAPMAN GRANT, MAJOR, U. S. ARMY *

INTRODUCTION

The Sphaerodactyl is little known to many collectors. He is hard to find, harder to catch and still harder to classify. That is, unless you enjoy working in the open and turning over innumerable rocks or scraping seeming acres of dead leaves. Hard to catch unless you can make up your mind to grab a fraction of a second sooner than the uncovered prize makes up his mind to dart; and he is a creature of quick decision and action. Hard to classify unless you have a large series to work with, thus avoiding the confusion of growth phases and sexual dichromatism. I know of no genus that more amply repays finding, catching and classifying than the sprightly little Sphaerodactyl.

The most valuable specific characters of these lizards at this place, using large series are:

1. Sexual dichromatism.
2. Keels or absence thereof on scales of underside.
3. Form and size of scales of snout.
4. Average adult size.
5. Color, pattern and proportions.
6. Color and pattern of newly hatched young.
7. Geographical and environmental distribution.

The male of all species has an escutcheon about five to seven scales long by ten wide, of very smooth, unkeeled scales on the lower belly, but not reaching the vent by five or six rows. It extends onto the thighs. It is obvious in even the smooth bellied species and makes sex determination easy. See Plate XX.

The egg is laid in loose dirt under or near the edge of a rock or under trash. Apparently only one is deposited at one place. The

* Major Grant has brought together a large herpetological collection while on foreign duty with the 65th Infantry in Porto Rico.

egg is nearly the size of the head so I believe a considerable time elapses between deposits. It is elliptical in outline. The shell is brittle in contrast to other lizard eggs of the vicinity. The unlaid egg can be clearly seen through the abdominal wall. The incubation period is about eight weeks.

The local name for the Sphaerodactyl is "Santa Lucia". Other names are too local to be considered.

I find no specific differences in the scale rows or labials which I have omitted in the descriptions. I have followed Barbour's "standard distance", i.e. the number of dorsal scales contained in the distance, snout to center of eye. I find the snout scales more specific and count the first row of large scales in front of eyes across snout and from this line to rostral.

Most of the species have circumscribed distribution. I believe that fortuitous distribution is exceptional. The forms are plastic and I believe that almost every island has a specific form.

I have drawn up two keys to supplement one another for the easy classification of the Porto Rican species and another one for interpolation in Dr. Thomas Barbour's key in his "Sphaerodactylus", Mem. Mus. Comp. Zoo., 1921.

It will be noted that the five new species of Sphaerodactyls herein described fall into a small subdivision of Dr. Barbour's key. This might suggest that a large series might show intergrades and that I have described variants of one or two species. I have large series and the lines of demarcation are definite and abrupt. There is no close similarity between the species and no great variation within the various species, as is more clearly shown in my keys than in the interpolation into Dr. Barbour's key. Furthermore, there is no close approximation to forms from other islands. Some writers have mistaken sexual dichromatism for variation.

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SQUAMATION KEY TO THE SPHAERODACTYLS OF PORTO RICO

A. Belly scales strongly keeled and pointed----------*S. klauberi*
AA. Belly scales smooth and rounded.
B. Scales on snout even in size, elongate and keeled----------*S. macrolepis*
BB. Scales on snout, except first two rows, elongate, even in size and keeled.
C. Scales on head strongly keeled---*S. danforthi*
CC. Scales on head weakly keeled ---*S. monensis*
BBB. Scales on snout hexagonal, not elongate.
C. Scales on snout keeled----------*S. nicholsi*
CC. Scales on snout smooth.
D. Scales on snout wider than long; twice as wide as those on head, uneven ----------*S. townsendi*
DD. Scales on snout hexagonal, regular----------*S. roosevelti*

COLOR, SIZE, MARKING AND LOCALITY KEY OF SPHAERODACTYLS OF PORTO RICO

A. Scapular pattern prominent.
(*a*) Scapular "mask" pattern vivid. Perfect tail tipped with white. Female with "target" head pattern; head of male plain. Sacral pattern on female only, if present, and greatly subordinated to scapular pattern. Body usually evenly spotted with brown scales in broken lines, more pronounced and larger in female. Adult average 60 mm. Not found in exceptionally dry or very humid situation. Porto Rico--*S. macrolepis*
(*b*) Scapular "mask" pattern less pronunced. Never pronounced white tip to tail. Female more pronounced head pattern and larger body marks than in *S. macrolepis.* Males speckled or red head; not plain as in *S. macrolepis.* Under side of head except in red-head males, black and white speckled ending abruptly at throat. Adult averages 50 mm. Culebra Island----------*S. danforthi*

(*c*) Scapular pattern large, but dull, tending to assume "spectacle" shape. Above gray; below white. No sexual dichromatism. Rows of brown dots on back. Males have swollen heads and necks. Male escutcheon reduced in size. Adult averages 50 mm. Mona Island.....*S. monensis*

AA. Scapular pattern reduced or wanting.

B. Very dark brown. Seldom any scapular pattern and then merely two light dots. Underside of tail yellow or orange, seldom brown. Belly brown or lemon. Adult averages 60 mm. Found in humid mountain situations.....*S. klauberi*

BB. Above gray or light brown. Underside white or gray.

C. Above light gray. Females with longitudinal dark and white stripes. Belly white. Adult averages 70 mm. Head large, heavy, obtuse. Found on SW corner of Porto Rico.....*S. roosevelti*

CC. Brown above, belly gray. Adult averages not over 50 mm.

D. White dots of scapular pattern usually present. Sacral pattern usually very clear. Tail usually lighter color than body. Adult averages 40 mm. Found on SW corner of Porto Rico.....*S. nicholsi*

DD. No trace of scapular pattern. Sacral pattern usually indicated. Practically no markings. Adult average 50 mm. Found on NE corner of Porto Rico and Caja de Muertos island.....*S. townsendi*

KEY FOR INTERPOLATION IN "SPHAERODACTYLUS", BARBOUR, 1921, Page 226, line 14.

e 2. Dorsals keeled and larger, no differentiated mid-dorsal zone.

* * * * * *

e 2. Five to seven scales "*1."

* * * * * *

f 1½. Five to 5½ scales, species large (70 mm.), striped lengthwise or plain ..... *S. roosevelti*

f 2. More than 5 scales.

g-1. Scales of belly keeled ..... *S. klauberi*

g-2. Scales of belly and chest smooth ..... *S. nicholsi*

g 1. Scales of chest keeled, belly smooth.

x 1. Scales on snout hexagonal and smooth ..... *S. townsendi*

x 2. Scales on snout elongate, even in size and keeled ..... *S. macrolepis*

y 1. Scales on head strongly keeled ..... *S. danforthi*

y 2. Scales on head weakly keeled ..... *S. monensis*

*1. ". . . scales" refers to the number of dorsal scales contained in length from snout to center of eye.

NOTE. A letter from Dr. Loveridge dated April 9, 1931, in answer to my query, states that *S. notatus* has chest smooth. It is therefore not close to any of the new species described herein and does not belong where shown in Dr. Barbour's key.

Sphaerodactylus roosevelti sp. nov.

TYPE: No. 864, male. Chapman Grant Collection. Near Parguera, Porto Rico; 29 Dec., 1930; collector, Chapman Grant.

DIAGNOSIS: The largest species of the region. Marked sexual dichromatism. Gray above, white beneath. Head heavy, salamander-like. Dorsals strongly keeled, belly smooth, feebly keeled collar.

HABITAT: The extreme southwestern corner of Porto Rico where I have found it only in dry, rotten logs. Vegetation scant.

PROPORTIONS: About 10 mm. longer than *S. macrolepis or S. klauberi*, and much heavier. The head blunt, almost salamander-like. Body thick and heavy, in which it differs from all the other species of Porto Rico. About 70 mm. in length.

SQUAMATION: Scales 5 in distance from snout to center of eye.

UPPER SIDE: Scales on snout smooth, hexagonal, changing between the eyes to slightly elongate and keeled, which continue to the head, all very even in size, changing suddenly at nape to large, very pointed, keeled, imbricated scales. Scales across snout in front of eye 16. This line to snout 9.

LOWER SIDE: Scales at point of jaw large, regular, followed by smaller rounded scales which suddenly enlarge into imbricated, round scales at neck, where there is occasionally a feebly keeled collar, then large, smooth, rounded belly scales.

ORBITAL SPINE: Prominent but not as long as head scales. Gray with a black tip.

COLOR AND MARKINGS: FEMALES: Above three white stripes, beginning, one between eyes and one behind each eye. Another beginning below each eye and all extending full length of body and tail. On head, stripes bordered with black. Brown between black stripes. On body and tail alternate gray and darker stripes each about three scales wide. Below white.

MALES: Above clear gray. Beginning at neck 5 faint yellowish stripes extend length of body and onto tail. Below white.

Newly hatched young not observed.

RELATIONSHIP: This species stands alone, due to its heavy proportions and lack of color. It differs greatly from *S. macrolepis, S. vincenti* and *S. monilifer*, the only heretofore described species with keeled chest scales.

REMARKS: Specimens taken, 4. Named in honor of Theodore Roosevelt, Jr., Governor of Porto Rico.

Sphaerodactylus nicholsi sp. nov.

TYPE: No. 912, male. Chapman Grant Collection. Near Ensenada, Porto Rico, 31 Dec., 1930; collector, Chapman Grant.

DIAGNOSIS: The smallest species, averages 40 mm. Scapular pattern reduced to white dots. Scales on snout hexagonal, keeled. All underside smooth. No sexual dichromatism. Brown above with irregular lines of brown dots. "V" shaped pattern at root of tail.

HABITAT: Found in the extreme southwestern corner of Porto Rico under stones in very dry, exposed localities. Vegetation scant.

PROPORTIONS: This is the smallest of the Porto Rico Sphaerodactyls, averaging 40 mm. The general proportions are similar to *S. macrolepis.*

SQUAMATION: Scales 6 in distance snout to center of eye.

UPPER SIDE: Scales at snout large, uneven, hexagonal, keeled (except those in contact with rostral), twice the size of head scales. On head, feebly keeled, small, regular, changing abruptly at neck to large, imbricated, keeled scales with posterior margins pointed. Scales across snout in front of eye 10. This line to snout 5.

LOWER SIDE: At point of chin a group of large, hexagonal, bead-like, smooth scales, followed by very even, hexagonal small ones changing into imbricated smooth scales near neck and then suddenly into large, imbricated, smooth scales which continue several rows beyond vent, all with posterior margins rounded.

ORBITAL SPINE: About one-half the length of head scales.

COLOR AND MARKINGS: Brown above with 2 or 4 irregular broken lines of dark brown dots. Scapular pattern reduced to 2 light spots, each completely encircled by a narrow, dark ring. Sacral pattern almost always very clear, formed by 2 black lines joining at base of tail, borded on the outside by a white "V". Tail frequently yellowish. Throat clear or dotted, belly clear gray, under side of tail usually light orange, sometimes gray speckled. Newly hatched young brown above without any markings. Lighter beneath, no markings. No sexual dichromatism in this species.

RELATIONSHIP: This species is unique because of its small size. It resembles *S. townsendi* in having large, coarse scales on the snout, but they are keeled in this species.

REMARKS: Specimens taken 57. Some contained mature eggs. Named in honor of John Treadwell Nichols. The nearest approach in appearance to heretofore described species is *S. vincenti,* Barbour, 1921, Plate 9 Fig. 3, which has scales half the size of *S. nicholsi* and a keeled collar.

Sphaerodactylus danforthi sp. nov.

TYPE: No. 1725, male, Chapman Grant Collection. 16 April, 1931, Culebra Island, Porto Rico; collector, Chapman Grant.

DIAGNOSIS: Pronounced sexual dichromatism. Adult averages 50 mm. Squamation as in *S. macrolepis.* Males less marked and females more than in *S. macrolepis.* The outstanding character is

the vividly speckled throat, black and white, stopping abruptly at neck.

HABITAT: Culebra Island and Louis Pena Key, Porto Rico.

PROPORTIONS: Similar to *S. macrolepis*, but averages 50 mm. *S. macrolepis* averages 60 mm.

SQUAMATION: Similar to *S. macrolepis*.

COLOR AND MARKINGS: MALES, RED-HEAD PHASE: Top and sides of head brick red, no markings. The red fades to brown in alcohol in a few days. Back, light brown with broken lines of fine dots extending on to tail. Underside nearly white to vent. Tail tinged with yellow and speckeled with brown. 16 out of 67, or 22 per cent of the males were of this phase. These embrace males of from medium to adult size. The red head is not an exclusive adult coloration.

MALES, SPECKLED-HEAD PHASE: 49 of 65 males, or 78 per cent had heads speckled above but without target pattern. Under side of head black and white speckled, half and half, ending abruptly at throat. Rest of body as in red-head phase.

FEMALES: The 60 females have a large target pattern on head, usually a round black spot near occiput with a longitudinal black oval around it. A scapular pattern, less pronounced than in *S. macrolepis*, is followed by a series of black blotches along the back, or more frequently by five lines of large brown dots. Throat speckled as in speckled headed males. Remainder of body as in males.

YOUNG: 62 young are olive brown above with pattern clearly indicated. Lighter beneath. A trace of white tail tip and faint scapular dots which in *S. macrolepis* are decidedly white.

RELATIONSHIP: In general proportions this species is closest to *S. macrolepis* from which it differs in smaller size; coarser, darker pattern; males of two color phases; the greater degree of sexual dichromatism and the usually vividly speckled throat with the speckling stopping abruptly at neck. It differs greatly from *S. vincenti* and *S. monilifer*, the only other heretofore described species with keeled collar.

REMARKS: Barbour, "Sphaerodactylus", 1921, Plate 6, Fig. 2 shows a good representation of a female *S. danforthi*. His Fig. 3 resembles a female *S. macrolepis*. I gathered about 30 eggs in loose dirt under heaps of dead vegetation. The incubation period is about 8 weeks. The young are very active. Specimens taken 157. I believe that further study will show that two species are included in the above, as the red head males have larger scales than the others

and there are large scaled females, but I have not as yet definitely separated them. The *Sphaerodactyl* on Culebrita Island does not agree with those described above. A large series may prove of interest. Named in honor of Dr. Stuart T. Danforth.

Sphaerodactylus klauberi sp. nov.

TYPE: No. 975, Male. Chapman Grant Collection. 11 Jan. 1931, El Yunque, Porto Rico; collector, Chapman Grant.

DIAGNOSIS: The only species in this region with keeled belly scales. Very dark brown above. Below dark brown or some shade of orange or yellow.

HABITAT: This species is found in the moist canyons of the higher mountains where the ground is saturated. It hides under stones or logs or is found within rotten logs.

PROPORTIONS: Similar in size and proportions to *S. macrolepis*, from which it differs in keeled belly scales and color.

SQUAMATION: Scales 6 in distance snout to center of eye.

UPPER SIDE: Scales at snout larger than on head, all pointed, elongated, keeled (except those bordering rostral), gradually enlarging at nape to the larger dorsals. Scales across snout in front of eyes 14, this line to snout 6.

LOWER SIDE: At point of chin, group of larger, even elongate, pointed, keeled (except those touching mental) scales, followed by similar smaller ones which gradually become larger towards belly. All elongated, keeled, imbricated and pointed, continuing for 5 or 6 scales below base of tail.

ORBITAL SPINE: One-half length of head scales. Not much larger than preceding prominent scales.

COLOR AND MARKINGS: Almost black above. Scapular pattern, if present, rudimentary and not obscured by dark back but reduced to two light, not white, dots. Under side dark, frequently tinted with a shade of lemon or orange. Under side of tail usually a deep orange but sometimes brown. Newly hatched young not observed. No sexual dichromatism in this species.

RELATIONSHIP: In general proportions this species is closest to *S. macrolepis*, from which it is easily distinguished by its keeled belly scales, absence of a distinct scapular pattern and by its very dark color.

REMARKS: Specimens taken 24. Differs widely from *S. goniorhynchus*, *S. fantasticus* and *S. microlepis*, the only other species with keeled bellies. Named in honor of Laurence M. Klauber.

Sphaerodactylus townsendi sp. nov.

TYPE: No. 1080, female. Chapman Grant Collection. 25 Jan., 1931, Cape San Juan, Porto Rico; collector, Chapman Grant.

DIAGNOSIS: No trace of scapular pattern. Sacral pattern usually indicated. Practically no markings. Average adult 50 mm. Scales on snout hexagonal, smooth, wider than long, uneven.

HABITAT: The promontories that jut out at the northeast extremity of Porto Rico and Caja de Muertos island. Vegetation, scant grass and cactus.

PROPORTIONS: Between the heavy *S. roosevelti* and the slender *S. macrolepis*. General proportions closest to *S. monensis* from which it differs in squamation and pattern.

SQUAMATION: Scales 6 in distance snout to center of eye.

UPPER SIDE: Scales at snout very large, uneven, smooth, hexagonal, wider than long, twice the diameter of those on head. On head, small, regular, elongated, keeled, gradually changing at neck to large, imbricated, keeled, pointed scales. Scales across snout in front of eyes 10. This line to snout 5.

LOWER SIDE: Scales at point of chin large, hexagonal, smooth, bead-like, followed by very even, hexagonal small ones, changing into imbricated scales near neck where there is occasionally a collar of feebly keeled scales, suddenly changing into large, imbricated, smooth scales which continue several rows below the vent. All imbricated scales with posterior margins rounded.

ORBITAL SPINE: Small, shorter than head scales, but prominent, since not preceded by large scales.

COLOR AND MARKINGS: Upper surface brown, lead gray in alcohol. Head minutely speckled but without pattern. No scapular pattern. Back uniform, or with a few broken rows of brown dots, frequently becoming distinct in the sacral region and joining at the base of the tail, forming a sacral pattern. Frequently a faint light line from the eye to the pelvis making the sacral pattern more distinct. Tail frequently with faint tinge of yellow. Chin faintly speckled, belly light gray or with a suggestion of orange. Newly hatched young solid brown above, lighter beneath. No sexual dichromatism in this species.

RELATIONSHIP: Closest to *S. monensis* from which it is clearly distinguishable by pattern and squamation. Differs greatly from the other keeled collar species.

Seventeen specimens from Caja de Muertos island averaged lighter underneath and fewer markings above. A large series may prove it to be a sub species.

REMARKS: Specimens taken 340. Named in honor of Charles Haskins Townsend.

Sphaerodactylus macrolepis Gunther

Synonymy omitted

DIAGNOSIS: Scales above strongly keeled; on snout, elongate, even, keeled. Below, smooth except for keeled collar. Scapular pattern vivid. Tail tipped with white. Sexual dichromatism. Average adult 60 mm.

HABITAT: Distributed in foci throughout Porto Rico except in localities of extreme drought or moisture. It is found under stones or piles of rubbish.

PROPORTIONS: Head small and pointed. Tail equal to length of head and body. Slender. About 60 mm. in length.

SQUAMATION: Scales 5 or 6 in distance, snout to center of eye.

UPPER SIDE: Scales at snout even, keeled (except those bordering rostral), elongate similar in shape and size to those on top of head which change suddenly at nape to the pointed, keeled, imbricated dorsals. Scales across snout in front of eyes 16, this line to snout, 7.

LOWER SIDE: At chin, a group of large, elongated scales followed by smaller ones, all feebly keeled to neck, where a collar of rounded keeled scales is followed by smooth, rounded belly scales.

ORBITAL SPINE: As long as head scale. Preceded by one or two prominent scales.

COLOR AND MARKINGS: Varies slightly, but is best known by its scapular pattern of a black blotch containing two livid white spots. Resembles a black mask. Back brown with six broken lines of dark brown scales to the tail, where they usually continue as irregular dark spots. The tip of a perfect tail is white, sometimes preceded by a black ring, but this marking is never regenerated and hence is rarely found in adults. Throat clear or dotted. Belly clear gray or yellowish. Underside of tail gray, brown or some shade of orange.

NEWLY HATCHED YOUNG: Black above, lighter beneath, with tip of tail white and two scapular white dots.

SEXUAL DICHROMATISM: Males: Head above, clear brown or finely speckled. Very rarely a faint trace of pattern. Spots on body smaller than on female. No sacral pattern. Speckled chin more frequent than in female.

FEMALES: Pronounced target pattern of a black spot near occiput surrounded by a longitudinal black oval. Rarely a female is seen without head pattern. Spots on body larger than on male. Some have sacral pattern. A few have finely speckled chins.

The dichromatism is not as pronounced as in *S. roosevelti* or *S. danforthi*. There is only a small percertage however, that might confuse a person unused to handling the species. After a short acquaintance the division into sexes can readily be made by color pattern without checking on the male escutcheon, which is, of course, clear and definite.

RELATIONSHIP: In general proportions this species is closest to *S. klauberi* and *S. danforthi*. The keeled belly scales and dark color of the former and the smaller size and different markings of the latter, make it very distinct.

REMARKS: Specimens taken 140. This species is best described and figured by Stejneger, 1904, Figs. 46–50. Schmidt, 1928 shows a female in his Fig. 26 A and a male in Fig. 26 B. He did not note the significance of the markings, attributing it to variation. Barbour, 1921, shows a female in Plate 6 Fig. 3. Fig. 2 resembles a female *S. danforthi*. Stejneger, 1904 shows a male in Fig. 51 and a female in Fig. 52. On Page 605 we find: "In the specimens collected in the white clay hills about three miles east of Ponce on April 16 (Nos. 27306–12) two similar types of coloration are also easily distinguishable, namely, those with uniform yellow heads without dusky markings but with the tail spotted like the back, and those with an unspotted ochraceous tail but a drab-colored head with a pattern, like Fig. 52, and longitudinal dusky marks on the back." Stejneger did not note that the two types of coloration corresponded with the sexes.

I believe that the above form is really *S. grandisquamis*, Stejneger, 1904, and that *S. macrolepis* is confined to St. Thomas and possibly other Islands. I hope to be able to prove this at a later date.

Sphaerodactylus monensis (Meerwarth)

1901 *Sphaeradactylus macrolepis a monensis* Meerwarth, Mitth. Naturh. Mus. Hamburg, XVIII, p. 20. Type locality, Mona Island; types, Hamburg Mus., Nos. 1207 a–b.

1904 *Sphaerodactylus monensis* (Meerwarth) Stejneger, U. S. Nat. Mus. p. 607.

1921 *Sphaerodactylus macrolepis* Gunther, Barbour, Mem. Mus. Comp. Zoo. p. 253.

1928 *Sphaerodactylus macrolepis* Gunther, Schmidt, N. Y. Acad. of Sci. Vol. X. Part I., p. 70–74.

1930 *Sphaerodactylus macrolepis* Gunther, Barbour. Zoologica, Vol. XI No. 4.

DIAGNOSIS: Medium size, 50 mm. Back gray, belly white. Scapular pattern distinct but pale. Sacral pattern crescent shaped. Belly smooth, chest feebly keeled. Male escutcheon much reduced. No sexual dichromatism. All scales on upper side of head are feebly keeled except for a patch between eyes which is strongly keeled.

HABITAT: Mona Island, Porto Rico.

PROPORTIONS: Similar to *S. townsendi* except large males have distended cheeks and necks. Differs in pattern and squamation.

SQUAMATION: More feebly keeled throughout than any other species in this region. Belly smooth, chest feebly keeled. Male escutcheon much reduced. First two rows of scales on snout hexagonal, smooth, wide, followed by elongated, keeled scales between eyes, followed by small, bead-like, feebly keeled scales on head which change abruptly at nape (beginning of scapular pattern) to pointed, imbricated, keeled dorsals. First row of large scales across snout in front of eyes averages 11½, this line to snout averages 4½.

ORBITAL SPINE: Short. Followed by one or two enlarged scales.

COLOR AND MARKINGS: Gray above; white beneath. Head pattern very faint olive markings on tan background. Dusky line forward from eye. Broken lines of brown scales on back. Scapular pattern with a distinct tendency to "spectacle shape" with neither the white nor black distinct; a very subdued pattern. Sacral pattern present, in form of a transverse crescent, points forward, dark line anteriorly bordered by light line posteriorly. More prominent than in any species of this region. Throat not dotted. No yellow under side of tail, except in specimens shrivelled in alcohol which turns many specimens brown. No sexual dichromatism. No young or eggs observed.

RELATIONSHIP: Unique in markings and keeling. Large males have the swollen neck shown by Barbour, 1921, Pl. 6, Fig. 1, *S. gilvitorques* Cope. It has no other similarity however. It differs from *S. macrolepis* in not having sexual dichromatism and in markings and color and specially in squamation of head which does not approximate *S. macrolepis*. It differs from *S. townsendi* in squamation and pattern. It is not close to any other species of this region. In a series it is unlikely to be confused with any other form.

REMARKS: Specimens taken, 51. This species is found under trash or objects on the ground. It is extremely active. I believe there may be two species on Mona Island.

Explanation of Plates XX–XXIV

Fig. 1. *Sphaerodactylus klauberi*, sp. nov.
Ventral aspect of male to illustrate escutcheon.
The escutcheon is found only on the males and makes sex determination easy. Note shape and position. The keeled belly scales of this unique species makes the escutcheon very prominent, but it is discernible in all species. This figure also illustrates the patch of fine scales in front of the hind leg. There is another patch behind the front leg. Common to all species.
6 × natural size.

Fig. 2. *Sphaerodactylus macrolepis* Gunther. Female. Note "target" pattern on head and prominent scapular "mask" pattern; coarse dots on dorsum and indication of sacral pattern.

Fig. 3. Same species. Male. Note plain head, lack of sacral pattern and finer dots on dorsum than on female.

Fig. 4. *Sphaerodactylus roosevelti* sp. nov. Female. Note shape of head and vivid unique markings.

Fig. 5. Same species. Male. No markings.

Figs. 2–5 all 1⅓ × natural size.

Fig. 6. *Sphaerodactylus danforthi* sp. nov. Underside of speckle-head male. Note prominent escutcheon scales on this smooth-bellied species. Note speckled throat and immaculate belly which are the same in female.

Fig. 7. Same species. Female. Note "target" pattern on head and coarse black spots on dorsum. Underside same as Fig. 6 except that there is no escutcheon on female.

Fig. 8. Same species. Male. Speckle-head phase. Note fine speckles and absence of head pattern; dorsal dots finer than on female.

Fig. 9. Same species. Red-head male. Note plain head, speckled back and absence of pattern.

Fig. 10. Same species. Red-head male. Underside. Note plain chin. The escutcheon can be plainly seen. Note larger scales on Figs. 9 and 10. This may prove to be a separate species, but to date I am unable to distiguish between the females.

Figs. 6–10 all 1¾ × natural size.

Fig. 11. *Sphaerodactylus nicholsi* sp. nov. Note comparative size; no head pattern; scapular pattern reduced, but sacral pattern prominent. No sexual dichromatism.

Fig. 12. *Sphaerodactylus townsendi* sp. nov. Note comparative size and absence of all save sacral pattern. No sexual dichromatism.

Fig. 13. *Sphaerodactylus klauberi* sp. nov. Note comparative size, dark color and absence of markings. No sexual dichromatism.

Figs. 11–13 all 1⅔ × natural size. Specimens are average.

PLATE XX.

PLATE XXI.

PLATE XXII.

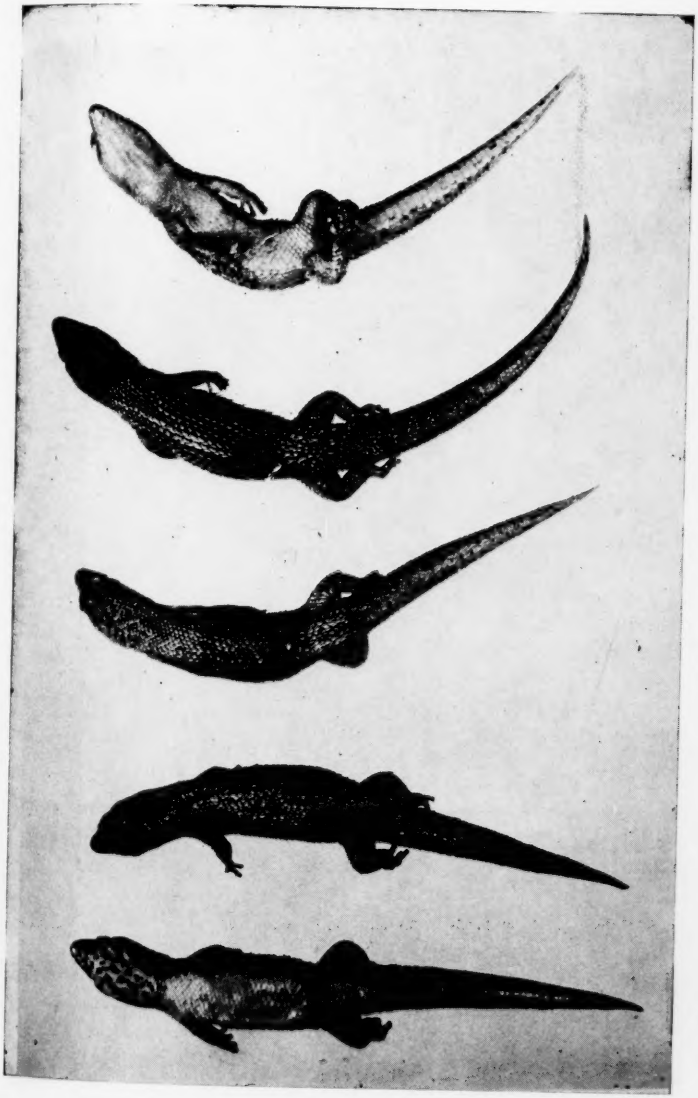

PLATE XXIII.

PLATE XXIV.

Fig. 14. *Sphaerodactylus monensis* (Meerwarth) Male. Note greatly reduced escutcheon and pure white color. Both sexes are white underneath.

Fig. 15. Same species. Male. Showing greatly enlarged head and neck. Note scapular pattern is not vivid and is in form of "spectacles".

Figs. 16–17. Same species. Females. Note no sexual dichromatism. Note sacral pattern peculiar to this species.

Figs. 14–17 all 2½ × natural size.

A REVISED LIST OF THE HERPETOLOGICAL FAUNA OF CULEBRA ISLAND

CHAPMAN GRANT, MAJOR, U. S. ARMY

Schmidt, 1928 gives the following nine amphibians and reptiles as comprising the Culebra Island herpetological fauna. After each name is added the number of specimens collected on the present trip.

1.	*Leptodactylus albilabris* (Gunther)	4
2.	*Eleutherodactylus antillensis* (R. & L.)	1
3.	*Anolis cristatellus* (see below)	0
4.	*Anolis stratulus* Cope	16
5.	*Anolis pulchellus* (D. & B.)	23
6.	*Amieva exsul* Cope	20
7.	*Mabuya sloanii* (see below)	0
8.	*Dromicus exiguus* Cope	0
9.	*Alsophis antillensis* (Schlegel)	28

The following species were taken and are added to the above list:

1.	*Sphaerodactylus danforthi* sp. nov. 1*	180
2.	*Anolis roosevelti* sp. nov. 2*	1
3.	*Typhlops jamaicensis* (Shaw)	10

Of the original list I have changed the following:

1.	*Mabuya sloanii* (Daudin) to *M. semitaeniatus* (Wiegmann) 3*	27
2.	*Anolis cristatellus* D. & B. to *Anolis cristatellus wileyi* sub sp. nov. 4*	41

This makes a total of 12 species now known to inhabit Culebra Island.

At the time of my visit, April 15–24, 1931, the island was extremely dry which accounts for the poor showing of *Salentia* and probably for the lack of *Dromicus exiguus* Cope which may have been aestivating.

I found *Anolis cristatellus wileyi* the only common reptile with *Sphaerodactylus danforthi* next in number. *Amieva exsul* was not common on Culebra, not seen on Louis Pena key, but was the most abundant form on Culebrita. *Anolis pulchellus* (D. & B.), *Anolis stratulus* Cope and *Amieva exsul* Cope showed no variation from their Porto Rican relatives.

1*, 2*, 3* and 4* described on pages 205, 219, 217 and 220 respectively in this number.

REESTABLISHMENT OF A SCINCID LOST SINCE 1837

CHAPMAN GRANT, MAJOR, U. S. ARMY

Mabuya semitaeniatus (Wiegmann)

Euprepes semitaeniatus, Wiegmann, Arch. f. Nat. p. 135------ 1837
Mabuya sloanii (Daudin) Stejneger, Report U. S. Nat. Mus. for 1902 p. 610---------- 1904
Mabuya sloanii (Daudin) Schmidt, N. Y. Acad. Sci. Vol. 10, Pt. 1, p. 121---------- 1928
Mabuya sloanii (Daudin) Barbour, Zoologica, p. 105---------- 1930
The type is No. 5290 in the Berlin Nat. Hist. Museum.

Wiegmann described *Euprepes* (*Mabuya*) *semitaeniatus* in 1837 from an unknown source. Since that time the few specimens obtained have been grouped with *M. sloanii* (Daudin) as synonymous. The difference in coloration was noted by Stejneger and Schmidt, but sufficient material was not at hand to prove the specific differences.

My collection contains six *Mabuyas* from Porto Rico which agree with Schmidt, 1928 Fig. 38 A in color and pattern. Counting his specimens this makes 8 from Porto Rico which are clearly *M. sloanii.*

I have just taken a series of 27 specimens from Culebra Island and the adjoining SW key, known as Louis Pena Key, and 35 from Mona Island. All agree exactly in color and pattern with Schmidt, 1928, Fig. 38 B in having the narrow median light line. This brings the total to 62 which agree in coloration.

My Porto Rico specimens have the back a uniform brown. Most of those from Culebra and Mona islands have a series of marks consisting of the center third segment of the scale being almost black. These marked scales spread from the lateral and dorsal dark bands, forming broken lines of triangular dots, more or less numerous along the back and clear down the tail in some specimens. The median line on the head is as light as the white dorso-lateral line in some from Mona and Culebra islands, but much darker in those from Porto Rico. The dorso-lateral light lines on the Porto Rico specimens are irridescent bluish along the body, whereas they are cream colored in the Culebra and Mona islands specimens.

I kept one female alive and she bore four young in from 36 to 48 hours between the first and last. The mother and young take insects, crickets, cockroaches and sowbugs. The young are colored like the adult except the tail which is white at the tip and lavendar nearly

to the body. This is reminiscent of the "blue tailed" skink of the middle west United States, *Eumeces guttulatus,* which it remained for Burt, 1928 to show was the young of *E. obsoletus* (B. & G.)

On Culebra and Mona islands the *Mabuya* or "Lucia" as the natives call it, is found among *Opuntia repens* Bello, at sea level just above the beaches and among the rocks on the hills. Some inhabit houses. They are tame, but show strength when held in the fingers. They make no attempt to bite.

In discussing *M. spilonota*, Wiegmann, from Jamaica and *M. sloanii* (Daudin); Stejneger 1904. p. 609, states: "Thus, separated supranasals and three pairs of nuchals seem to predominate in *M. spilonota*, while supranasals in contact and two nuchals belong to the eastern form". (*M. sloanii* and *M. semitaeniatus.*) These observations are borne out by my series of which 43 have the supranasals in contact and 18 only have them separated. Of nuchals, 2 have 1–1; 15 have 2–1; 31 have 2–2; 9 have 2–3; 2 have 3–3; 1 has 1–6 and two are mangled.

I notice that most specimens, and especially the young, have a pronounced transluscent pineal spot, sometimes pit, at the posterior part of the interparietal scale. One freak specimen has two small scales at this point which resemble occipitals.

The separation of *M. semitaeniatus* from *M. sloanii* rests on the constant color and pattern difference. The specimens in my collection I believe reestablish the name *Mabuya semitaeniatus* (Wiegmann) for the Culebra and Mona island species.

Photograph by Captain Frederick V. Edgerton, U. S. A.

Explanation of Plate XXV

Mabuya semitaeniatus Wiegmann.
Note head pattern.
Four young were born in captivity. Their tails are lavendar with white tips.
1⅓ × natural size.
Photo by Capt. Frederick V. Edgerton, U. S. A.

PLATE XXV.

A NEW SPECIES AND TWO NEW SUB-SPECIES OF THE GENUS ANOLIS

CHAPMAN GRANT, MAJOR U. S. ARMY

Anolis roosevelti sp. nov.

TYPE: No. 1884, male, Chapman Grant Collection, Culebra Island, Porto Rico, 22 April, 1931; collector, Chapman Grant.

DIAGNOSIS: A giant *Anolis* of the *A. cuvieri* type. Top of head deeply grooved. Ear opening triangular. Lower loreal row small. Head more pointed than in *A. cuvieri* viewed both from side and above.

HABITAT: Culebra island.

PROPORTIONS: Similar to *A. cuvieri* except head which is more slender and pointed. Larger than any specimen of *A. cuvieri* of which I can find any record.

DESCRIPTION:	*A. cuvieri*	*A. roosevelti*
Top of head	flat	deeply grooved.
occipital	barely noticeable	fairly prominent
lower loreal row	largest	small
ear opening	small, oval	large, triangular
dorsal ridge	50 spines	70 small spines
scales on chin	keeled	not keeled
rows between rays	3 to 4	5
loreal surface	vertical	sloping
Measurements of Sschmidt's "largest specimen", 1928.		
length of head	43 mm	56
breadth of head	26	33
arm	60	91
leg	100	142
body	135	160

A further difference is that if the loreal ridge were projected to intersect the line of the cutting edge of the upper jaw they would intersect in *A. cuvieri* beyond the end of snout and in *A. roosevelti* at end of snout.

The supraocular semicircles leave a deep groove from the occiput

of the males are uncommon on Porto Rico, but predominate on the keys just east of Porto Rico and on Mona Island.

Anolis cristatellus wileyi subsp. nov.

HABITAT: Culebra Island and adjoining keys.

TYPE: No. 1885, Chapman Grant Collection, Culebra Is., P. R. collector, Chapman Grant. 15 April 1931.

DIAGNOSIS: Similar to *Anolis cristatellus cristatellus* except that the throat fan of the male has an edging of not less than one-half the radius of very deep orange. The females have the entire patch deep orange. This is constant.

During a ten day collecting trip I preserved 41 specimens, handled many more and observed hundreds of *Anolis cristatellus wileyi*. There was no exception to the wide, deep orange edge of the fan. The difference between this and the Porto Rican species is striking. High tail fins are more numerous than on Porto Rico. A preserved specimen resembles *Anolis cristatellus cristatellus*.

Named in honor of Grace Olive Wiley.

Anolis cristatellus cooki subsp. nov.

HABITAT: Brea Point, S. W. Porto Rico.

TYPE: No. 780. Chapman Grant Collection, Brea Point, P. R., collector, Chapman Grant. 28 Dec., 1930.

DIAGNOSIS: Similar to *Anolis cristatellus cristatellus* except that the entire throat fan is chocolate color, and a light line beginning on the upper labials extends above the front leg, thence diagonally down to the groin.

The southern side of Punta Brea, SW of Guanica is desert-like. A few shrubs and cactus grow among coral slabs. In an area 100 by 500 yards in extent I found a few small, slender *cristatellus* with uniform chocolate colored fans. The body color was nearly white. I attach no significance to the white color or the small size, since I took only five specimens. There were no intermediate forms and no specimens inhabited the area between this little colony and the north side of the point where the bull headed, orange and green fan *Anolis cristatellus cristatellus* was found. In alcohol these specimens remain lighter than the Porto Rico form, but the peculiarity of the fan is lost to a great extent.

Named in honor of Dr. Melville T. Cook.

This little colony forms a link between *Anolis cristatellus cristatellus*, D. & B. and *Anolis monensis* Stejneger. It resembles the

to near the snout. At center of ridges, groove is half as deep as wide. The canthus rostralis and semicircle have a groove between them half way to snout. Two median ridges from line with fore part of orbits to near snout inclose a deep groove and form two lesser grooves with the projection of supraocular semicircles. Thus at level with fore part of orbits there are five distinct grooves.

Fin on tail very high. Third from distil ray twice as long as depth of tail. The fourth proximal ray as long as depth of tail.

Scales on side of tail larger than in *A. cuvieri* which in turn are larger than in *A. ricordii.*

At 5th ray from base of tail:	*A. cuvieri*	*A. ricordi*	*A. roosevelti*
longitudinal rows	10–14	16–20	10
vertical rows	3–4	5–6	5

A comparison with Stejneger's 1904 Figs. 85–86 shows that *A. roosevelti* differs from *A. ricordii* greatly in the grooves on the head.

Color: Living specimen; brownish gray with two light lines on each side. One from ear to groin, the other from shoulder to groin. A distinct light spot on temple. Eyelids yellow. Fan gray except lower rear quarter which is light yellow. Tail yellowish brown. Underside whitish.

Named in honor of Theodore Roosevelt Jr., Governor of Porto Rico.

One specimen taken.

TWO NEW SUB-SPECIES OF ANOLIS CRISTATELLUS

Anolis cristatellus cristatellus Dumeril and Bibron, on Porto Rico runs the gamut of all the color changes and patterns known or imagined. After eight months of intimate contact with them, during which time I have preserved well over 300, picked up and handled many more and watched literally thousands, I still occasionally see a pattern or color combination new to me. The females are the ones that show the most marked diversity. One thing, however, I consider of sub-specific value. That is the colors of the throat fan. Unfortunately these do not last well in alcohol. The colors fade and the fan becomes stiff and shriveled. On Porto Rico the fan varies from a uniform light olive green throughout to a light green center edged with orange. The orange edge seldom equalling one half the radius of the fan and usually only a narrow edging. In some cases the light orange extends up on the sides of the neck. High tail fins

latter in having the oblique lateral light line and in having the fan a uniform color, but lacks the specific transocular dusky line. I have noted in handling many fresh or living *Anolis monensis* that the fan is almost invariably of one color—orange yellow. Occasionally one is seen with a suggestion of green in the center of the fan and occasionally an orange fan is seen, but never the deep orange of the Porto Rico form (*A. c. c.*) At Playa Sardinera on Mona Island I have seen specimens with a lavendar metallic luster on the head and on the tail fin. The belly of *Anolis monensis* averages much brighter deeper yellow than the other forms. Stejneger, 1904, p. 646 in describing *Anolis monensis* says: "... a distinct transocular, dusky line. In many specimens there is a distinct white line from shoulder to groin. The color of the dewlap can not be made out in alcoholic specimens, but there are indications that it is different from that of *Anolis cristatellus.*" This description was based on 14 specimens. It is fully supported by the 185 specimens in my collection. Barbour, 1930 admits the validity of *Anolis monensis.* Schmidt, 1928 considered it synonymous with *Anolis cristatellus cristatellus.*

Explanation of Plates XXVI–XXVII

Fig. 1. *Anolis roosevelti* sp. nov. Note pointed snout and high occipital region.
Fig. 2. *Anolis cuvieri* Merrem. Note blunt snout and low head.
Figs. 1 and 2. Life size.
Fig. 3. *Anolis roosevelti* sp. nov. Note deep grooves on head; prominent occipital; pointed snout.
Fig. 4. *Anolis cuvieri* Merrem. Note flat head; absence of visible occipital scale.
Figs. 3 and 4. 1¼ × natural size.

PLATE XXVI.

1 2

STUDIES ON CACAO

I. ACTINOMYCETES ON CACAO BEANS

R. Ciferri, Santiago, Dominican Republic.

A paper on Actinomycetes or ray-fungi which causes the "mould odor" or "ground odor", or, according to Fellers (**11**), "Actinomyces odor" on dry cacao beans was published in the German Language (**2**), and later new observations were published in the Italian language (**3**). These two papers will be briefly summarized at this time in order to make this series of studies more complete.

In spite of the fact that arthrospores, or true spores, or fragments of the mycelium of Actinomycetes are normally present on dry fermented and unfermented cacao beans, the peculiar taste is not perceptible until the full development of the ray-fungi, e. g., after 52–76 hours of incubation in moist environment. The presence of the microorganisms may be revealed also by the presence of a superficial white dust. Its development is active at 20–25° C., maximum from about 30° C. to 40° C., but in the last case, the taste is mixed musty and rancid, in the other being from musty to musty-ground. Cultures from washing water of cacao seeds in carrot agar and in Czapek-Waksman agar revealed from 1110 to 3760 Actinomycetes for each gram of cacao beans, with an average of 2063 Actinomycetes on eight determinations.

The species most frequently isolated is the *Actinomyces albus* Krainsky emend. Waksman and Curtis, according the description of Waksman (**28**), in a slightly distinct variety (var. *alpha* Cif.). *Actinomyces griseus* Kr. and three other not identified species or forms were present, but scarcer.

In the opinion of the writer, this microorganism is passively carried from the soil to the shell of the cacao pod by the winds and the rainfall. Washing the bark of healthy pods the writer obtained from 36 to 408 colonies of Actinomycetes per gram of fresh pod, with an average of 195 colonies (six determinations). From the air of a cacao estate Actinomycetes were present in the proportions of 3, 9, 8, and 14 per cent of the colonies developed. But the most active agents for carrying spores of Actinomycetes are the men who break the shells of the cacao pods, extracting the masses of cacao beans and isolate the seeds from the rachis. If the beans are fermented, another source

of contamination is the fermenting boxes, from which Actinomycetes were isolated. The Actinomycetes are diffused also by the fermentation liquor, containing from 5 to 11 ray-fungi for each gram of the liquor, with an average of 7 from four determinations. The fermentation humour was inoculated until 4906 colonies of *Actinomyces albus* were obtained for each c. c.; samples were taken each three days and the colonies enumerated. On the 15th day the number decreased to 3550. The conclusion was that the *Actinomyces* live but in an inactive form in the fermentation liquor. The same humour, freshly taken from the fermentation boxes, sterilized and inoculated with 13780 germs per c. c. at the 15th day contained 14070 ray-fungi per c. c.

Previously the writer (**4**) studied the mouldy taste of the wine, finding that the ray-fungi are living on rotten wood of the casks and tuns (less well on unaltered wood), and may communicate easily to the wine the peculiar taste *. According to Fellers (**11**), the bitter taste and the Actinomyces odor of the milk is caused by the *Actinomyces griseus* Kr. Zedfield attributed the same taste of the eggs to the ray-fungi. The mouldy taste of Javanese tobacco sent from Java to Europe in good condition, according to Gandrup (**13**) is caused by unidentified species of Actinomycetes.

The complete prevention of the contamination of cacao beans is impossible, from the practical standpoint, but may be reduced to a minimum by: (1) avoiding the contamination by soil; (2) isolating rotten or wounded pods, chiefly the pods damaged by the bird (*Picumnus* [*Centurus*] *striatus*) and by fruit-eating bats, from healthy pods; (3) avoiding the introduction in the mass of cacao beans of leaves, small branches, and portions of shell of the cacao tree or pod; (4) rigorous cleaning and disinfection of the fermentation boxes; (5) avoiding the mixture of cacao beans from rotten or wounded pods with beans from healthy cacao pods.

Also it may be noted that unfermented cacao beans are poorer in Actinomycetes than the fermented ones (from 320 to 1230 colonies for each c. c. of washing water, with an average of 776 per c. c., as obtained from six determinations), and, of course, unfermented cacao is less susceptible to the mouldy taste than the fermented.

From the technologic standpoint, this moulding is of course, very distinct from the true moulding, but of a very reduced importance, as the contamination is limited to the external surface of the cacao

* The Actinomycetes causing the mouldy taste of the wine was identified as the *Streptothryx Sanninii* Cif., but according to the most accepted generic nomenclature, it is a species of *Actinomyces* and very similar to *A. albus*.

beans. From the commercial standpoint, it may be observed that the responsibility of the farmer is very limited, and the growth in number of the Actinomycetes, as well as the development from the arthrospores or resting spores being effected during shipment.

II. FUNGOUS FLORA OF DRY CACAO BEANS

Two types of cacao mouldings are recognizable on dry fermented and unfermented Dominican cacao, namely: (1) Cacao moulding which gives a peculiar and disagreeable odor known as "mould odor" or "Actinomyces odor". This odor is caused by many species of the genus *Actinomyces*. Colonies of such common moulds as Mucorales and Hyphales may or may not be present. Strongly contaminated cacao frequently appears superficially covered with a whitish delicate dust which is composed of colonies and more or less fragments of the ray-fungi. (2) Cacao moulding without any particular odor, but showing a more or less luxuriant growth of mould fungi (Mucorales and Hyphales), composed of colonies which are blackish, brownish or green-bluish, rarely whitish to yellowish or reddish in color. Very frequently, the two types of moulding are associated, as the ray-fungi are almost constantly present on dry cacao beans, but the "Actinomyces odor" is clearly perceptible only if cacao seeds are intensively contaminated.

In a previous paper (**2**), the writer referred on the first type of cacao moulding. The most frequent and important ray-fungus is the *Actynomyces albus* Krainsky emend. Waksm. & Curt., with a local but scarcely distinguished variety *alpha* Cif.; also *A. griseus* Krainsky is present. The following moulds were isolated: *Rhizopus arrhizus* Fisch., *R. nigricans* Ehrenb., *Macrosporium commune* Rabenh., *Coniothecium effusum* Corda, *Botrytis vulgare* Fries, *Aspergillus fumigatus* Fres., *A. niger* V. Tiegh., *A. elegans* Gasp., *Fusarium* sp., *Spicaria lateritia* Cif.

The purpose of the present investigation, carried out during the years 1926 and 1927 was: (1) the study of numerical distribution of the mould spores on dry fermented and unfermented healthy cacao beans, as well as on moulded cacao beans; (2) the identification of isolated moulds; (3) the study of the comparative growth of the most frequent moulds on pasteurized fermented and unfermented cacao beans.

Scattered notices about moulds of cacao and cacao moulding are found in the literature on cacao, but the first satisfactory determinations of moulds were obtained from the studies of Dr. Thom of

cultures made by Dr. Schwarz from Gold Coast (Accra type) cacao (**26**). He found that several forms of *Aspergillus niger* and *A. flavus* group, were present in practically every phase of the culture work; *A. tamarii* was encountered in the beans taken from near the surface of the mass. He found also abundant Mucoraceace, chiefly species of *Rhizopus*, a few yeasts, and aerobic spore-forming bacteria, including bacteria of the *mesenteric group.**

Reinke (**23**) isolate 142 strains of Aspergilli from cacao, of which the most frequent were *Aspergillus flavus* and *A. niger* (75 per cent), *A. Sydowii* and *A. tamarii* (50 per cent); also *A. repens, A. terreus, A. carbonarius, A. versicolor* var. *flavipes, A. candidus, A. giganteus, A. ochraceus* and *A. versicolor.* From Haitian samples, he isolated *A. niger, A. tamarii, A. carbonarius* and *A. flavus.*

Green, brown, and black *Aspergilli,* six *Mucorales,* seven *Penicilli,* three *Cladosporium, Cephalothecium, Fusarium, Botrytis* and a Sphaeropsidaceous fungus were isolated from cacao beans of many African and American countries by Busse, Henneberg and Zeller (**1** bis).

EXPERIMENTAL PROCEDURE

Samples of fermented, unfermented and mixed (fermented and unfermented) cacao beans, of about one pound each, were taken from the store houses of the cacao farmers and traders of Moca, Santiago, La Vega, San Francisco de Macorís, Sánchez and Samaná, and carefully packed. As soon as possible, 25 beans from each sample were washed with 100 c.c. of sterile, distilled water. Holding firmly with a sterile forceps, each cacao bean was thoroughly scrubbed with a sterile stiff stencil brush. Duplicate plate cultures on Bacto prume agar were made, using five, ten, fifteen and twenty drops and one c.c. of thoroughly agitated wash water. To inhibit the growth of Schizomycetes, the substratun was mercurized to 1/10.000 (adding 1 c.c of one per cent solution of mercury chloride to 100 c.c. of prune agar), following the method of De Rossi (**10**). Each set of ten plates was incubated in the laboratory at ordinary room temperature (24–32° C.) during three days, and all apparently different colonies of fungi were isolated for later identification. Numbered stock cultures were subdivided in five groups, according

* During the publication of this paper, I knew of the isolation and study of many other fungi isolated from cacao beans. Sartory A., Sartory R. and Meyer J. (Ann. Mycol. **28** (5–6): 362–362. 1930) isolated a species of *Aspergillus (A. halophilus)* from sample of cacao damaged by the contact with sea water and subsequently stored under warm condition I was informed of a few papers on this subject published in the English colonies of Africa, but without knowledge of the content, as well as of the bibliographical reference.

to the results of a preliminary identification, namely: (1) Aspergilli forms, (2) Penicilli forms, (3) species of Mucorales, (4) mould fungi other than the preceding, (5) Saccharomycales and Torulopsidaceae (ascosporic and anascosporic yeasts).

The cacao used was the common cacao (so called Sánchez type), chiefly derivated from Calabacillo variety-group crosses. Samples marked *A* to *G* were taken from 1926 crop, and samples marked *H* to *L* from 1927 crop. The locality and quality of samples was the follow:

Sample A: mixed healthy cacao from Moca
Sample B: unfermented healthy cacao from Moca.
Sample C: fermented healthy cacao San Francisco de Macorís.
Sample D: mixed healthy cacao from San Francisco de Macorís.
Sample E: mixed healthy cacao from La Vega.
Sample F: mixed healthy cacao from Samaná.
Sample G: mixed moulded cacao from Moca.
Sample H: mixed healthy cacao from Santiago.
Sample I: unfermented healthy cacao from San Francisco de Macorís.
Sample J: fermented healthy cacao from San Francisco de Macorís.
Sample K: mixed moulded cacao from Samaná.
Sample L: mixed moulded cacao from Sánchez.

NUMERICAL DISTRIBUTION OF MOULD FUNGI

The numerical distribution of spores of mould fungi per cacao bean (colony counts) * was as follows:

Sample	Per cent Mucorales of	Per cent of Aspergilli	Per cent of Penicilli	Per cent of other moulds	Total number of colonies
A	32	41	21	6	700
B	46	39	7	8	1,400
C	17	66	15	2	1,300
D	56	38	5	1	1,700
E	39	44	13	4	1,900
F	54	41	5	0	4,200
G	48	36	15	1	89,500
H	39	47	13	1	3,000
I	60	21	16	3	4,700
J	40	54	5	1	600
K	67	18	15	0	67,100
L	74	24	2	0	38,900
Average	48	39	10	3	17,917

AVERAGES

Average of mould spores on mixed healthy cacao (samples A, D, E, F and H): 2300.
Average of mould spores on fermented healthy cacao (samples C and J): 950.
Average of mould spores on unfermented healthy cacao (samples B and I): 3050.
Average of mould spores on mixed moulded cacao (samples G, K, and L): 65270.

* True and anascosporic yeasts, besides as scarce as 0, 1 per cent or less, will be studied in another section of this paper.

IDENTIFICATION OF THE MOULD FUNGI

Group (1) Aspergilli. The systematic and nomenclature of Thom and Church (1) has been followed. Colonies, growing on Czapeck's solution agar, were incubated in the Laboratory at room temperature. In view of the fact that the isolated *Aspergillus* strains, are not specialized on cacao, but are more or less saprophytic forms, the identification was frequently limited to the forms-group only.

Strains N. 12, 15, 31, 56 and 60 belong to the *Aspergillus niger* group, and probably, should be determined as *A. niger* V. Tiegh., an exceedingly variable form. This species is present in almost all cultures in Petri dishes, and is frequently the most abundant mould.

Strains N. 17, 26, 29, 34, 40 and 48 belong to the *A. fumigatus* group, but I have never seen the perithecial forms. This mould is as frequent as the preceding group-species.

Strains 18, 19, 42, 46 and 59 belong to the *A. glaucus* group, and this species is very frequent, but only in certain samples (A, B, D, G, K, L).

Strain N. 38 and 39 belong to the *A. tamarii* group, and is perhaps identic with *A. tamarii*. As in the preceding, this form is frequent only in certain samples (samples *F, K* and *L*).

Strains 22, 24 and 52 belong to the *A. nidulans* group; strains N. 22 and 24, isolated from the sample G, are probably identical with the true *A. nidulans.*

Strains N. 30, 36, 44 and 68 belong to the *A. flavus* group. Strains of this group are as frequent as the *A. niger* and *A. fumigatus.*

Strain N. 67 belong to the *A. versicolor* group. A rare form isolated only from the sample *D.*

Strain N. 85 belong to the group of *A. candidus.* As in the preceding, but from the sample *J.*

Group (2) Penicilli. The study published by Biourge has been followed, and the cultures grown on Raulin-Diercky and on Hayduk solutions, etc., are made according the procedure described in Biourge's monograph and at room temperature.

Strains 32, 54 bis, 58 and 82.—*Penicillium leucopus* (Pers.) Biourge. This form, probably corresponding to *Penicillium glaucum* or *P. crustaceum* Auct. pl., is the one most frequently seen on cultures in Petri dishes, but never as abundant as the blackish Aspergilli.

Strains N. 43 and 64.—*P. notatum* West. An unfrequent form, isolated from the samples D and G.

Strain N. 51.—A form allied with *P. luteum* (Zuk.) Thom (fide Biourge) isolated from the sample H, and here very frequent.

Strain N. 33.—This strain is definite, but very doubtfully, as the *P. roseum* Link, a classic but from the modern point of view, not clearly definite species. It is not considered in Biourge's monograph. It was isolated from the sample E only once.

Strains N. 77 and 80.—These strains are identical with or allied to *P. candidum* Roger (nec Link, fide Biourge), and were isolated from samples J and K. Found also as indefinite, small, arachnoid colonies on moulded cacao beans.

Group (3) Mucorales. Species of this order were studied and classified following the technic and the systematic and nomenclature of Lendner (**18**).

Strains N. 14, 20, 24, 53 and 65.—These strains, isolated from the samples A, C, D, G, H and K, were referable to *Rhizopus nigricans* Ehr.

Strains N. 35, 65 and 83.—*Rhizopus arrhizus* Fisch, isolated from the samples G, K, and L.

Strains N. 22 bis, 75 and 87.—These strains must be referred to *Mucor mucedo* L., a mould as frequent as the *Rhizopus nigricans* in all moulded cacao beans.

Strain N. 74.—This strain, isolated from the sample I, is closely allied or, probably, identic with *Mucor racemosus* Fres.

Group (4) Mould fungi other than Aspergilli, Penicilli and Mucorales. The species of Hyphales isolated from cacao beans were identified following the systematic treatment of this group by Lindau (**20**) and Ferraris (**12**). Cultures were made using many solid media, chiefly Bacto prune agar, peptonized potato agar and carrot agar.

Strains 22, 30, 58 and 71.—All these strains must be referred to *Spicaria lateritia* Cif., one of the saprophytic fungi most universally distributed in the Dominican Republic. It is, also, one of the most frequent contaminaters of cultures. Conidia are present in the air, in water, in soil, etc. It forms large and beautiful colonies, orange or lateritic-red in color, on partially burned wood during the rainy seasons or in moist places.

Strains N. 13, 21, 74 and 79.—All these strains are referable to *Cephalosporium acremonium* Corda, a mould almost as frequent as *Spicaria lateritia*.

Strains N. 39 and 50.—Another largely distributed mould, referred to *Trichothecium roseum* Link.

Strain N. 25.—This strains must be referred to a new species of *Helminthosporium*, a saprophyte described as *H. cacaophilum* Cif., very distinct from *H. theobromicolum* Cif. and *H. theobromae* Ture. Found only in the sample B.

Strain N. 45.—Belongs to *Macrosporium commune* (Rabenh.) Sacc., as conventionally understood. This is an unfrequent mould, isolated twice from sample A.

Strains N. 33, 63 and 73.—This mould was isolated three times from samples B, E, and G, but is probably more common. It is not different from *Pullularia pullulans* (De By.) Berkh., commonly called *Dematium pullulans* De Bary.

Strains N. 37 and 47.—Belongs to *Alternaria tenuis* Nees, and was isolated twice from samples G. and H. About the 50 per cent of the colonies isolated from the sample H are composed of this fungus.

Strain N. 41.—*Catenularia fuliginea* Saito, a rare species found only in the sample F.

Strain N. 65.—For this strain I am describing a new species of the genus *Dendryphium* (*D. congestum* Cif., n. sp.). It was found in the sample L, growing in a single colony.

Strain N. 38.—This strain is of doubtful identification; apparently it belongs to *Coniothecium effusum* Corda. It was found in a single colony from the sample C.

Strain N. 66.—Found in a single colony from cultures derivated from sample K. It was referred to a new genus and new species, *Blastoconium tropicum* Cif., n. gen. et n. sp.

Strain N. 79.—Isolated from sample B and doubtfully referred to *Hormodendron pallidum* Oudem.

Strain N. 55.—*Fusarium sarcochroum* (Desm.) Sacc., or an allied form, isolated from sample K.

Strain N. 78.—This strain must be referred to a slightly different variety of *Fusarium zonatum* (Sherb.) Wollenw., but its systematic position is doubtful. It was isolated from sample G.

GROWTH OF MOULDS ON CACAO BEANS

With a few exceptions, the isolation of a mould from cacao beans does not demonstrate the possibility of a luxuriant growth on the same, or, in other words, that the fungus must be considered as one of the causes of cacao moulding.

The inoculations were made by spraying a suspension of the conidia (from cultures in Petri dishes) in distilled sterilized water with a small atomizer on: (1) fermented uncut cacao beans, (2) fermented cut beans, (3) unfermented uncut cacao beans, (4) unfermented cut beans. Before the inoculation, the four samples of cacao beans were washed with 0.2 per cent solution of mercury bichloride and repeatedly re-washed with sterilized destilled water, and then enclosed in four ample dessiccators partially filled with water, during four days. After the water imbibition by cacao beans, each sample was divided in twenty small samples, all of which was inoculated with one of the isolate moulds, and enclosed in a common sterilized drinking-glass, closed by a photographic plate glass and sealed with paraffine. The set of eighty glasses were kept during thirty days in incubation at the laboratory temperature, and then opened and the growths of moulds observed as appearing to the naked eye. Of the isolated moulds, only twenty forms, appearing as the most frequent, were inoculated. The results are summarized as follows:

(The sign O signifiy no growth; ? doubtful growth; + scarce development; + + abundant development; + + + very abundant development).

Mould	Fermented cacao		Unfermented cacao	
	Cut beans	Uncut beans	Uncut beans	Uncut beans
Aspergillus niger	+ + +	+ + +	+ + +	+ + +
Aspergillus fumigatus	+ + +	+ + +	+ + +	+ + +
Aspergillus glaucus	+	+	+ +	+ +
Aspergillus tamarii	+	?	+	?
Aspergillus nidulans	0	0	+	+
Aspergillus flavus	+	+	+ +	+ +
Aspergillus versicolor	0	?	?	?
Aspergillus candidus	0	0	0	?
Penicillium leucopus	+	+	+	+
Penicillium notatum	0	0	0	0
Penicillium luteum	0	0	?	+
Penicillium roseum	0	0	?	?
Penicillium candidum	0	?	+	+
Spicaria lateritia	0	0	0	?
Cephalosporium acremonium	0	0	0	0
Trichothecium roseum	0	0	?	0
Pullularia pullulans	0	0	0	0
Alternaria tenuis	0	0	?	+
Rhizopus nigricans	+ +	+ +	+ + +	+ + +
Rhizopus arrhizus	+	+	+	+
Mucor mucedo	+ +	+ + +	+ +	+ + +
Mucor racemosus	0	0	?	?

DISCUSSION OF THE RESULTS

The number of strains and mould forms isolated from cacao beans should be summarized as follows:

(1) Aspergilli: 27 strains, 8 forms.

(2) Penicilli: 10 strains, 5 forms.
(3) Mucorales: 12 strains, 4 forms.
(4) Mould others than the preceding: 24 strains, 14 forms.
These strains derived from the following cacao beans samples:

Sample A: 10 forms.	Sample G: 16 forms.
Sample B: 11 forms.	Sample H: 10 forms.
Sample C: 9 forms.	Sample I: 8 forms.
Sample D: 11 forms.	Sample J: 9 forms.
Sample E: 8 forms.	Sample K: 16 forms.
Sample F: 10 forms.	Sample L: 12 forms.

Or, in relation to the quality of cacao, an average of:

Mixed healthy cacao: 10 forms.
Unfermented healthy cacao: 10 forms.
Fermented health cacao: 9 forms.
Mixed moulded cacao: 15 forms.

As one may expect, the fermented healthy cacao beans are the poorest in forms of moulds, and moulded cacao the richest. Mixed and unfermented cacao are equally rich in forms of moulds, and intermediary between the preceding samples.

Aspergilli are the most frequent moulds, and the group is the richest in forms. Penicilli and Mucorales are almost of the same importance. The following species are normally present in samples of cacao beans: *Aspergillus niger; A. fumigatus; A. flavus; A. glaucus; Penicillium leucopus: Rhizopus nigricans; Mucor mucedo; Spicaria lateritia; Cephalosporium acremonium.* Mucorales are the most abundant as to number of spores, then Aspergilli, Penicilli and, last, other Hyphales.

These results agree well with the observation made by Schwarz in Gold Coast on Accra cacao beans, and in relation to the isolated forms, with the preliminary experiments of the writer.

SUMMARY

The writer refers to the results of isolation from and inoculation of fermented and unfermented cacao beans of mould fungi. A new genus and two new species of Dematiaceae are described.

MYCOLOGICAL OBSERVATIONS AND DESCRIPTIONS OF STRAINS

Strain N. 25.—*Helminthosporium cacaophilum* Cif., n. sp. (Description from cultures.)

At the room temperature, the colonies develop easily; colony 5 days old is from 20 to 45 mm. in diameter. It is composed of a well developed system

of myceliar hyphae, without clearly differentiated conidiophores, and a few scattered conidia. The colony is, at first, white and tufted, then sub-lanose and developed in concentric but not well defined rings, varying from light-gray to dull-gray, with black shades. Mycelium abundant, composed of densely but irregularly branched hyphae, septate, containing droplets and refringent corpuscula, generally from 2 to 3.5 mmm. in diameter. Short lateral sub-erected myceliar branches 15–25 mmm. in length, may produce a few conidia, acrogenous or pleuro-acrogenous, inserted on small teeth. Conidia brown to grayish, from 5 to 13-septate, generally 7–10-septate, ellipsoid to ovoid, free and more or less rounded, basal end sub-acuminate, 65 to 94 mmm. in length, 10 to 15.5 mmm. width. Isolated from unfermented healthy cacao beans from Moca, Dominican Republic.

Strain N. 63.—*Dendryphium (Brachycladium) congestum* Cif., n. sp. (Description from cultures).

At room temperature, this fungus grows readily covering almost all the surface of the Petri dish. The colonies are flattened, at first black-greenish, then blackish, smooth, opaque, with a very poor aerial development. The mycelium is brownish, septate, densely branched, and developed abundantly only under the surface of the solid substratum, 2–3 mmm. in thickness. The conidiophores emerge from the substrata, but are sub-erect to prostrate, more or less straight, with a few septa, unbranched or scarcely branched, 20–50 mmm. by 2–3.5 mmm. The conidia are normally acrogenus, very rarely acro-pleurogenous, isolated or, more frequently, from 2 to 6-chained, 3–5-septate, smoky, clearly narrowed at the septa, from elliptic to ovoid, 14–33 by 4.58 mmm. Isolated from mixed moulded cacao beans from Sánchez, Dominican Republic.

Strain N. 66.—*Blastoconium* Cif., n. gen. (Hyphales, Dematiaceae, Phaerdictyae, Coniothecieae).

Similar to the genus *Coniothecium* Corda, but with sterile hyphae torulose-moniliform, from which each single isolated element (chlamydospore) may reproduce itself by budding. As in the genus *Coniothecium*, true conidiophores are absent, and the conidia are transversally and longitudinally septate.

Blastoconium tropicum Cif., n. sp. (Description from cultures).

Develop easily at room temperature. The colonies are, at first very similar to the colonies of the *Pullularia pullulans*, repeating the *Dematium*-like stage of most of the Dematiaceae; the growth of the underground part is more active than the superficial growth. The colonies are smoky to blackish, then black, smooth, humid. The mycelium is composed of branched hyphae, densely septate, at first narrowed at the septa, then producing by budding one or two lateral daughter cells. The daughter cells may separate and reproduce by budding or not, in the last case forming two cells more or less of the same size. Successively, the number of cells constituting the chain increases and branches, and at the same time a number of blastospores become deeper in color while the membrane increases in thickness. The final result is the presence of monilioid or torulose chains, composed of spheric or sphered, elliptic, ovoid, cylindric or irregularly-shaped elements, normally unseptated, rarely transversally septate, very variable in size, from 4

to 15 mmm. in diameter or in length. A few monilioid hyphae may be produced only on the surface of the solid substratum (exceptionally on liquid media) one isolated conidium, apparently generated from one aerial bud of the terminal chlamydospore, clearly distinct from the mother cell. The conidium is irregular in shape, more frequently elliptic or sub-cylindric, from 1-septate to 3-septate, but in many cases with a longitudinal irregular septum; the size of the conidia is 6–17 by 4–12 mmm. Isolated from mixed moulded cacao beans from Samaná, Dominican Republic.

This genus, like *Dematium* or *Pullularia*, probably represents the most primitive and imperfect stage of development of unknown Dematioid fungi.

According the deeseription, *Coniothecium glumarum* Sacc. (Syll. fung., Vol. XIV, p. 1092, 1899) found on *Phragmites communis* in Hungary, must be referred to this genus as BLASTOCONIUM GLUMARUM (Sacc.) Cif., n. comb. This species was described as having torulose-septate hyphae, and the microconidia are probably not other than the young bud-cells of the chlamydospores.

Strain N. 55.—*Fusarium ? sarcochroum* (Desm.) Sacc. Sect. *Latcritium* Woll. Microconidia trichothecioid, 1–3 septate, 12–25 by 4–10 mmm.; macroconidia 3–6 septate, 25–52 by 3–6 mmm.

Strain N. 78.—*Fusarium ? zonatum* (Sherb.) Woll. Sect. *Elegans* Woll., Subsect. *Oxysporum* Woll., Ser. *Pallens* Woll. Sporodochial confluent gelatinous stroma; microconidia very abundant, 4–10 by 2–4 mmm.; macroconidia 32–64 by 3–6 mmm. Probably a form of the species.

III. CACAO MOULDING

This study, made in combination with previous reports on this same subject, was performed in order to determine: (1) the environmental factors and conditions influencing the development of mould fungi, chiefly in relation to (*a*) moisture content of cacao beans; (*b*) moisture absorption and loss of the same; (*c*) temperature; (2) the determination of the critical point of moulding; (3) the prevention of moulding.

As in the preceding, the studies were made both on fermented and unfermented Dominican cacao, Sánchez type.

Preliminary observation having indicated that the most important role is played by moisture content of cacao beans, this factor was studied most accurately. At the same time, a few observation were made for determination of many constants of Dominican cacao, such as average dry weight, specific weight and size of beans.

ORIGIN OF THE SAMPLES

The origin of the samples and the type of cacao beans was the following:

Sample N. 1.—Mixed cacao from Moca (marked S. B.).
Sample N. 2.—Mixed cacao from Moca (marked E. P.).
Sample N. 3.—Fermented cacao from Moca (marked E. N. A.).
Sample N. 4.—Fermented cacao from Moca (marked E. N. A.).
Sample N. 5.—Unfermented cacao from Moca (marked E. N. A.).
Sample N. 6.—Unfermented cacao from Moca (marked E. N. A.).
Sample N. 7.—Fermented cacao from La Vega (marked E. G. G.).
Sample N. 8.—Fermented cacao from La Vega (marked E. G. G.).
Sample N. 9.—Unfermented cacao from La Vega (marked E. G. G.).
Sample N. 10.—Unfermented cacao from La Vega (marked E. G. G.).
Sample N. 11.—Unfermented cacao from Bonao (marked J. J.).
Sample N. 12.—Unfermented cacao from Bonao (marked J. J.).
Sample N. 13.—Unfermented cacao from San Cristóbal.
Sample N. 14.—Mixed cacao from San Cristóbal.
Sample N. 15.—Fermented cacao from San Francisco de Macorís (marked J. M. A.).
Sample N. 16.—Fermented cacao from San Francisco de Macorís (marked B. J. R.).
Sample N. 17—Unfermented cacao from San Francisco de Macorís.
Sample N. 18.—Unfermented cacao from San Francisco de Macorís.
Sample N. 19.—Unfermented cacao from Pimentel.
Sample N. 20.—Mixed cacao from Pimentel.
Sample N. 21.—Unfermented cacao from Villa Rivas.
Sample N. 22.—Mixed cacao from Villa Rivas.
Sample N. 23.—Unfermented cacao from Samaná.
Sample N. 24.—Mixed cacao from Samaná.

NORMAL MOISTURE CONTENT AND DRY WEIGHT OF CACAO BEANS

This constant was determined by taking the actual weight of samples of 100–500 cacao beans, and drying at 100–105°C.

ACTUAL WEIGHT, DRY WEIGHT AND MOISTURE CONTENT OF CACAO BEANS

Sample No.	Average of	Actual weight gr.	Moisture content per cent	Dry weight gr.	Observations
1.....	100 seeds....	1, 068	19	0, 865	
2.....	100 seeds....	1, 059	21	0, 837	
3.....	500 seeds....	0, 949	15	0, 807	
4.....	500 seeds....	0, 936	14	0, 805	
5.....	500 seeds....	1, 020	18	0, 836	
6.....	500 seeds....	1, 059	19	0, 858	
7.....	200 seeds....	0, 970	16	0, 815	
8.....	200 seeds....	0, 945	15	0, 803	
9.....	200 seeds....	1, 081	20	0, 865	
Average....	2, 800 seeds..	1, 010	19	0, 832	General average
Average....	200 seeds....	1, 064	20	0, 851	Average of mixed cacao beans
Average....	1, 400 seeds..	0, 922	15	0, 808	Average of fermented cacao beans
Average....	1, 200 seeds..	1, 053	19	0, 820	Average of unfermented cacao beans

SPECIFIC WEIGHT OF CACAO BEANS

This constant was determined using the pycnometric method. First observations were made by filling the pycnometer with mercury, but the great difference in the specific weight of mercury and that of cacao beans gave many difficulties. The following measurements were made using graduate cylinders filled with distilled water, having observed that the absorbed water does not affect the determinations, if they are made sufficiently rapid. Of course, the precision of this specific weight is reduced.

SPECIFIC WEIGHT OF CACAO BEANS (SAMPLE OF 6 BEANS EACH)

	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 5
	0, 991	0, 974	0, 891	0, 890
	0, 983	0, 972	0, 890	0, 890
	0, 986	0, 968	0, 890	0, 893
	0, 987	0, 966	0, 887	0, 895
	0, 988	0, 973	0, 884	0, 894
	0, 986	0, 974	0, 883	0, 890
	0, 990	0, 974	0, 884	0, 885
	0, 984	0, 975	0, 887	0, 886
	0, 988	0, 976	0, 888	0, 887
	0, 986	0, 972	0, 886	0, 890
Average....	0, 987	0, 971	0, 887	0, 890

Average of mixed cacao.....	0, 979
Average of fermented cacao.....	0, 887
Average of unfermented cacao.....	0, 890

The differences between the specific weight of samples Nos. 1 and 2 composed of mixed cacao, are probably caused by the different contents of moisture (undetermined).

SIZE OF CACAO BEANS

Fifty unselected cacao beans from each sample were measured; the figures of table express the maximum length, maximum breadth and the maximum thickness.

SIZE OF CACAO BEANS

Sample N. 1 (mixed cacao).

Length mm.:	16	17	18	19	*20*	21	22	23	24	25	
Frequency N.:	1	1	7	8	10	7	8	5	1	2	=50
Breadth mm.:	10	11	*12*	13	14	15	16				
Frequency N.:	3	4	17	13	9	3	1	=50			
Thickness mm.:	4	5	6	7	8	*9*	10	11	12	13	
Frequency N.:	1	2	9	8	11	12	3	3	0	1	=50

Sample N. 2 (mixed cacao)

Length mm.:	16	17	18	19	20	*21*	22	23	24	25	
Frequency N.:	1	0	2	2	8	13	10	5	7	2	=50
Breadth mm.:	10	11	*12*	13	14	15	16	17	18	19	
Frequency N.:	2	5	15	14	10	3	0	0	0	1	=50
Thickness mm.:	4	5	6	*7*	8	9	10	11			
Frequency N.:	2	4	6	16	14	5	2	1	=50		

Sample N. 3 (fermented cacao)

Length mm.:	16	17	18	19	*20*	21	22	23	24	25	26	
Frequency N.:	1	3	7	10	19	17	17	13	6	4	3	=100
Breadth mm.:	7	8	9	10	11	*12*	13	14	15	16	17	
Frequency N.:	1	0	0	4	10	30	24	27	7	0	2	=100
Thickness mm.:	3	4	5	6	*7*	8	9	10	11	12	13	
Frequency N.:	1	2	14	22	32	13	4	6	3	2	1	=100

Sample N. 5 (unfermented cacao)

Length mm.:	16	17	18	19	*20*	21	22	23	24	25	26	27	28	29	30	
Frequency N.:	2	4	2	13	19	14	9	9	13	11	1	2	0	0	1	=100
Breadth mm.:	9	10	11	12	*13*	14	15	16	17	18						
Frequency N.:	2	2	11	22	27	18	11	4	1	2	=100					
Thickness mm.:	4	5	6	*7*	8	9	10	11	12							
Frequency N.:	1	5	22	29	22	11	8	1	1	=100						

Average of the samples N. 1 and 2 (mixed cacao)

Length mm.:	16	17	18	19	20	*21*	22	23	24	25	
Frequency N.:	2	1	9	10	18	20	18	10	8	4	=100
Breadth mm.:	10	11	*12*	13	14	15	16	17	18	19	
Frequency N.:	5	9	32	27	19	6	1	0	0	1	=100
Thickness mm.:	4	5	6	7	*8*	9	10	11	12	13	
Frequency N.:	3	6	15	24	25	17	5	4	0	1	=100

These figures may be summarized as follows:

Mixed cacao: 16–25 by 16–19 by 4–13 mm.. most frequently 21 by 12 by 8 mm.

Fermented cacao: 16–26 by 7–17 by 3–13 mm., most frequently 20 by 12 by 7 mm.

Unfermented cacao: 16–22 by 9–18 by 4–12 mm., most frequently 20 by 13 by 7 mm.

WATER IMBIBITION OF CACAO BEANS

Three samples of 120 cacao beans were submersed in distilled water at 40° C., superficially dried using bibulous paper and weighed after 24 and 48 hours of immersion.

Sample N. 14 (mixed cacao)

Initial weight	gr. 1, 311 = 100
After 24 hours	gr. 1, 550 = 118
After 48 hours	gr. 1, 550 = 118

Sample N. 15 (fermented cacao)

Initial weight	gr. 1, 139 = 100
After 24 hours	gr. 1, 306 = 115
After 48 hours	gr. 1, 306 = 115

Sample N. 17 (unfermented cacao)

Initial weight	gr. 1, 228 = 100
After 24 hours	gr. 1, 478 = 120
After 48 hours	gr. 1, 478 = 120

LOSS IN WEIGHT OF CACAO BEANS EXPOSED TO THE SUNSHINE

Five samples of fermented cacao and five samples of unfermented cacao, varying from 10 to 25 kg. each, were exposed to the direct sunshine 11 hours; 7 hours the first day (from 9 A. M. to 4 P. M. August 26, 1927) and 4 hours the second day (from 8 A. M. to 11 A. M. August 27, 1927). These days were very warm, the temperature varying from 24 to 35° C. and from 24 to 34° C. Losses in weight are expressed in percentages.

PER CENT OF LOSS IN WEIGHT OF CACAO BEANS EXPOSED TO THE SUNSHINE

Hours	Sample No. 3	Sample No. 4	Sample No. 7	Sample No. 8	Sample No. 5	Sample No. 5	Sample No. 6	Sample No. 9	Sample No. 10	Sample No. 24	Average for hour	
											Fermented beans	Unfermented beans
1st	0, 37	0, 28	0, 33	0, 45	0, 38	0, 38	0, 79	0, 48	0, 80	1, 10	0, 36	0, 71
2nd	0, 35	0, 14	0, 14	0, 26	0, 16	0, 16	0, 32	0, 35	0, 49	0, 68	0, 21	0, 40
3rd	0, 35	0, 20	0, 04	0, 27	0, 19	0, 13	0, 29	0, 25	0, 31	0, 48	0, 20	0, 29
4th	0, 29	0, 10	0, 21	0, 24	0, 17	0, 17	0, 24	0, 25	0, 35	0, 41	0, 21	0, 28
5th	0, 27	0, 12	0, 07	0, 25	0, 12	0, 06	0, 20	0, 14	0, 18	0, 10	0, 15	0, 13
6th	0, 16	0, 05	0, 02	0, 08	0, 09	0, 10	0, 08	0, 08	0, 07	0, 00	0, 04	0, 06
7th	0, 00	0, 00	0, 00	0, 04	0, 00	0, 04	0, 00	0, 00	0, 03	0, 00	0, 00	0, 01
(Night)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)
8th	0, 00	0, 00	0, 18	0, 08	0, 11	0, 14	0, 10	0, 06	0, 05	0, 00	0, 13	0, 07
9th	0, 28	0, 18	0, 16	0, 23	0, 15	0, 14	0, 27	0, 12	0, 28	0, 52	0, 20	0, 27
10th	0, 20	0, 13	0, 10	0, 19	0, 13	0, 08	0, 07	0, 12	0, 12	0, 26	0, 13	0, 13
11th	0, 14	0, 10	0, 10	0, 11	0, 14	0, 10	0, 05	0, 15	0, 12	0, 11	0, 10	0, 11
Total	2, 42	1, 31	1, 34	2, 19	1, 64	1, 50	1, 50	1, 99	2, 71	3, 66	1, 78	2, 27

(The increase in weight during the night, if any, was not considered, and the weight was calculated on the basis of the 7th hour).

These results are graphically expressed in the diagram of plate XXVIII.

WATER VAPOR IMBIBITION OF CACAO BEANS

Ten samples of cacao beans (five of fermented cacao and five of unfermented cacao), composed of 20 seeds each, were exposed to saturated water vapor (100 per cent, or absolute humidity) at the temperature of the Laboratory (about 24 to 32° C.) during five days. For this purpose, the samples were enclosed in two moist chambers. The increase in weight is expressed in per cent of the initial weight.

PER CENT OF INCREASE IN WEIGHT OF CACAO BEANS EXPOSED TO THE SATURATED WATER VAPOR

Sample No.	First day	Second day	Third day	Fourth day	Fifth day	Total
4	0, 00	0, 47	0, 32	0, 01	0, 00	0, 80
7	0, 00	0, 24	0, 25	0, 06	0, 01	0, 56
8	0, 00	0, 26	0, 27	0, 01	0, 00	0, 62
15	0, 00	0, 46	0, 34	0, 06	0, 00	0, 63
16	0, 00	0, 41	0, 34	0, 01	0, 00	0, 56
Average of fermented beans	0, 00	0, 37	0, 30	0, 03	0, 01	0, 63
17	0, 00	0, 22	0, 35	0, 00	0, 00	0, 56
18	0, 00	2, 12	0, 65	0, 00	0, 00	2, 77
19	0, 00	0, 20	0, 37	0, 00	0, 00	0, 57
21	0, 00	0, 33	0, 65	0, 01	0, 00	1, 00
23	0, 00	2, 38	0, 59	0, 00	0, 00	2, 95
Average unfermented	0, 00	1, 07	0, 52	0, 00	0, 00	1, 57

These figures are represented in the graph of the plate XXIX.

WATER VAPOR ABSORTION OF CACAO BEANS DRIED AT DIFFERENT TEMPERATURES

Two samples of cacao beans (one of fermented and one of unfermented cacao), composed of 20 beans each, were dried until of constant weight at different temperatures, ranging from 40° C. to 100° C., then exposed to the saturated water vapor (in a moist chamber) during ten days, and weighted. The moist chamber was situated, as in the preceding experiment, in the laboratory. The increase in weight at the end of the experiment is expressed in per cent as related to dry weight.

PER CENT OF INCREASE IN WEIGHT OF CACAO BEANS DRIED TO DIFFERENT TEMPERATURES AND EXPOSED TO SATURATED WATER VAPOR

FERMENTED CACAO (SAMPLE NO. 15)

Day	Drying temperature degrees C.							Total
	40	50	60	70	80	90	100	
First	0, 51	0, 49	0, 28	0, 11	0, 13	0, 09	0, 08	1, 69
Second	0, 29	0, 27	0, 19	0, 18	0, 09	0, 11	0, 09	1, 22
Third	0, 28	0, 13	0, 12	0, 09	0, 10	0, 10	0, 09	0, 91
Fourth	0, 01	0, 04	0, 05	0, 02	0, 06	0, 03	0, 05	0, 26
Fifth	0, 00	0, 01	0, 00	0, 02	0, 01	0, 05	0, 02	0, 11
Sixth	0, 01	0, 00	0, 02	0, 01	0, 00	0, 02	0, 05	0, 10
Seventh	0, 00	0, 02	0, 00	0, 02	0, 01	0, 02	0, 03	0, 10
Eighth	0, 00	0, 00	0, 00	0, 00	0, 01	0, 01	0, 01	0, 03
Ninth	0, 00	0, 00	0, 00	0, 02	0, 00	0, 00	0, 01	0, 03
Tenth	0, 00	0, 01	0, 00	0, 00	0, 01	0, 00	0, 01	0, 03
Total	1, 10	0, 97	0, 66	0, 47	0, 42	0, 43	0, 44	4, 48

UNFERMENTED CACAO (SAMPLE NO. 17)

Day	Drying temperatures degrees C.							Total
	40	50	60	70	80	90	100	
First	0, 23	0, 20	0, 21	0, 18	0, 15	0, 12	0, 13	1, 22
Second	0, 12	0, 10	0, 20	0, 12	0, 15	0, 16	0, 10	0, 95
Third	0, 09	0, 11	0, 19	0, 13	0, 09	0, 10	0, 09	0, 80
Fourth	0, 11	0, 12	0, 09	0, 06	0, 08	0, 10	0, 09	0, 65
Fifth	0, 07	0, 05	0, 10	0, 08	0, 08	0, 11	0, 14	0, 73
Sixth	0, 02	0, 06	0, 09	0, 09	0, 07	0, 12	0, 17	0, 62
Seventh	0, 05	0, 07	0, 08	0, 10	0, 07	0, 03	0, 12	0, 52
Eigth	0, 06	0, 05	0, 11	0, 09	0, 08	0, 06	0, 08	0, 53
Ninth	0, 04	0, 08	0, 09	0, 03	0, 01	0, 05	0, 05	0, 35
Tenth	0, 08	0, 04	0, 05	0, 07	0, 08	0, 05	0, 00	0, 35
Total	0, 88	0, 88	1, 21	0, 95	0, 86	0, 90	0, 97	6, 62

These results referred also in the clivoids of plate XXX.

DAILY VARIATION OF WEIGHT OF CACAO BEANS AS RELATED TO THE AVERAGE OF AIR HUMIDITY

Specimens of 2 kg. each of both fermented and unfermented cacao beans were taken from the samples N. 3 and 5, and weighted three times per day, at 7 A. M., at 1 P. M. and at 5 P. M. The average of weights were divided in classes of 10 gr. each, from 2000 gr. to 2080 gr. These averages were compared with the average of daily relative humidity, taken three times at the same hours. The results were the following:

Unfermented cacao (Sample N. 5)

Weight gr.															
2000 to 2010	Humidity %	69	75	77	80	81	82	83	84	85	87	88	90	91
	Frequency N.	1	1	2	3	2	2	4	5	1	1	5	2	7
	Humidity %	92	94	95	96	97	100							
	Frequency N.	7	1	3	4	2	1							

Weight gr.

2011 to	Humidity %	63	69	72	76	80	83	87	88	91	95	96	
2020	Frequency N.	1	1	1	3	1	1	3	1	5	2	3	
2021 to	Humidity %	72											
2030	Frequency N.	1											
2031 to	Humidity %	60	62	64	69	83							
2040	Frequency N.	1	1	1	1	2							
2041 to	Humidity %	64	69	76	87								
2050	Frequency N.	1	1	1	1								
2051 to	Humidity %	75	76	83	87	91							
2060	Frequency N.	1	1	1	1	2							
2061 to	Humidity %	62	69	73	83	96							
2070	Frequency N.	1	1	1	1	1							
2071 to	Humidity %	76											
2080	Frequency N.	1											

Fermented cacao (Sample N. 3)

2000 to	Humidity %	60	63	69	72	75	76	77	80	81	82	83	85	87
2010	Frequency N.	1	3	2	3	1	1	4	1	3	4	1	1	5
	Humidity %	88	90	91	92	94	95	96	97	100				
	Frequency N.	4	2	3	1	3	1	1	2	1				
2011 to	Humidity %	69	72	76	80	83	87	91	95	96	97			
2020	Frequency N.	1	2	1	1	3	1	2	2	4	2			
2021 to	Humidity %	76	77	91	95									
2030	Frequency N.	1	2	1	1									
2031 to	Humidity %	62	64	69	76	80	87							
2040	Frequency N.	1	2	3	1	1	2							
2041 to	Humidity %	62	64	69	72	76	77	91						
2050	Frequency N.	1	2	1	2	1	1	1						
2051 to	Humidity %	69	73	76	77	83	95	96	97					
2060	Frequency N.	1	2	1	1	1	1	1	1					

It should be noted that the cacao beans were exposed in the open air, during several days and nights.

These results are represented in the diagram of plate XXXI.

HOURLY RECORD OF THE VARIATIONS IN WEIGHT OF CACAO BEANS

Two samples of 500 gr. each of fermented and unfermented cacao beans were taken and left to the open air. During 24 consecutive

hours, and each 15 minutes, the samples were weighted and the relative humidity recorded. The results were as follows:

HOURLY RECORDS OF VARIATIONS IN WEIGHT OF CACAO BEANS AND VARIATIONS OF RELATIVE HUMIDITY

Fermented Cacao Sample No. 3				Unfermented Cacao Sample No. 5			
Hour	Weight gr.	Per cent variations	Relative humid.	Hour	Weight gr.	Per cent variations	Relative humid.
6 p. m.	500, 0	+ 0, 00	91	6 p. m.	500, 0	+ 0, 00	92
6, 15 p. m.	500, 2		96	6, 15 p. m.	500, 1		92
6, 30 p. m.	500, 4		96	6, 30 p. m.	500, 1		91
6, 45 p. m.	500, 6		96	6, 45 p. m.	500, 2		91
7 p. m.	500, 8	+ 0, 16	96	7 p. m.	500, 3	+ 0, 06	91
7, 15 p. m.	501, 0		96	7, 15 p. m.	500, 4		91
7, 30 p. m.	501, 3		96	7, 30 p. m.	500, 5		91
7, 45 p. m.	501, 5		96	7, 45 p. m.	500, 5		91
8 p. m.	501, 7	+ 0, 34	96	8 p. m.	501, 2	+ 0, 24	91
8, 15 p. m.	501, 8		96	8, 15 p. m.	501, 7		96
8, 30 p. m.	501, 9		96	8, 30 p. m.	502, 1		96
8, 45 p. m.	502, 0		96	8, 45 p. m.	502, 2		96
9 p. m.	502, 3	+ 0, 46	96	9 p. m.	502, 4	+ 0, 48	96
9, 15 p. m.	502, 3		96	9, 15 p. m.	502, 5		96
9, 30 p. m.	502, 4		96	9, 30 p. m.	502, 6		95
9, 45 p. m.	502, 5		96	9, 45 p. m.	502, 8		95
10 p. m.	502, 6	+ 0, 52	96	10 p. m.	502, 9	+ 0, 58	95
10, 15 p. m.	502, 7		96	10, 15 p. m.	503, 1		95
10, 30 p. m.	503, 0		95	10, 30 p. m.	503, 4		95
10, 45 p. m.	503, 0		95	10, 45 p. m.	503, 9		95
11 p. m.	503, 2	+0, 64	95	11 p. m.	504, 5	+ 0, 90	95
11, 15 p. m.	503, 4		95	11, 15 p. m.	504, 7		95
11, 30 p. m.	503, 6		95	11, 30 p. m.	504, 9		91
11, 45 p. m.	503, 7		95	11, 45 p. m.	505, 7		95
12 p. m.	503, 8	+ 0, 76	95	12 p. m.	505, 9	+ 1, 18	95
12, 15 p. m.	504, 1		91	12, 15 p. m.	506, 1		95
12, 30 p. m.	504, 2		91	12, 30 p. m.	506, 3		95
12, 45 p. m.	504, 3		91	12, 45 p. m.	506, 5		95
1 p. m.	504, 5	+ 0, 86	96	1 a. m.	506, 7	+ 1, 34	95
1, 15 p. m.	504, 7		91	1, 15 a. m.	506, 9		95
1, 30 p. m.	505, 1		82	1, 30 a. m.	507, 2		95
1, 45 p. m.	505, 1		91	1, 45 a. m.	507, 3		95
2 p. m.	505, 1	+ 1, 02	95	2 a. m.	507, 7	+ 1, 54	95
2, 15 p. m.	505, 1		95	2, 15 a. m.	508, 0		95
2, 30 p. m.	505, 1		96	2, 30 a. m.	508, 1		95
2, 45 p. m.	505, 3		95	2, 45 a. m.	508, 1		95
3 p. m.	505, 3	+ 1, 06	95	3 a. m.	508, 7	+ 1, 74	90
3, 15 p. m.	505, 3		95	3, 15 a. m.	508, 7		90
3, 30 p. m.	505, 3		95	3, 30 a. m.	508, 8		90
3, 45 p. m.	505, 4		95	3, 45 a. m.	508, 9		90
4 p. m.	505, 4	+ 1, 08	95	4 a. m.	509, 0	+ 1, 80	90
4, 15 p. m.	505, 5		95	4, 15 a. m.	509, 1		90
4, 30 p. m.	505, 5		95	4, 30 a. m.	509, 3		90
4, 45 p. m.	505, 5		95	4, 45 a. m.	509, 3		90
5 p. m.	505, 4	+ 1, 08	95	5 a. m.	509, 3	+ 1, 86	91
5, 15 p. m.	505, 4		95	5, 15 a. m.	509, 3		90
5, 30 p. m.	505, 4		95	5, 30 a. m.	509, 7		90
5, 45 p. m.	505, 5		95	5, 45 a. m.	509, 9		90
6 p. m.	505, 5	+ 1, 10	95	6 a. m.	509, 9	+ 1, 98	90
6, 15 p. m.	505, 6		95	6, 15 a. m.	509, 9		90
6, 30 p. m.	505, 7		95	6, 30 a. m.	509, 9		95
6, 45 p. m.	505, 6		95	6, 45 a. m.	509, 9		95
7 p. m.	505, 2	+ 1, 04	95	7 a. m.	510, 1	+ 2, 02	95
7, 15 p. m.	504, 8		96	7, 15 a. m.	509, 3		91
7, 30 p. m.	504, 7		96	7, 30 a. m.	508, 9		91
7, 45 p. m.	504, 6		96	7, 45 a. m.	508, 1		84
8 p. m.	504, 5	+ 0, 90	96	8 a. m.	507, 4	+ 1, 48	83
8, 15 a. m.	504, 2		96	8, 15 a. m.	507, 4		91
8, 30 a. m.	504, 0		91	8, 30 a. m.	506, 9		91
8, 45 a. m.	503, 9		91	8, 45 a. m.	505, 3		91
9 a. m.	502, 9	+ 0, 58	92	9 a. m.	505, 6	+ 1, 12	96
9, 15 a. m.	502, 7		84	9, 15 a. m.	505, 1		96
9, 30 a. m.	500, 9		92	9, 30 a. m.	504, 8		96
9, 45 a. m.	500, 5		84	9, 45 a. m.	504, 5		96
10 a. m.	500, 2	+ 0, 04	84	10 a. m.	503, 7	+ 0, 74	96
10, 15 a. m.	499, 5		84	10, 15 a. m.	503, 4		96
10, 30 a. m.	499, 1		84	10, 30 a. m.	502, 9		96
10, 45 a. m.	498, 4		92	10, 45 a. m.	501, 9		96

HOURLY RECORDS OF VARIATIONS IN WEIGHT OF CACAO BEANS AND VARIATIONS OF RELATIVE HUMIDITY—Continued

Fermented Cacao Sample No. 3				Unfermented Cacao Sample No. 5			
Hour	Weight gr.	Per cent variations	Relative humid.	Hour	Weight gr.	Per cent variations	Relative humid.
11 a. m....	497,4	— 0,52	92	11 a. m..	501,0	+ 0,20	96
11,15 a. m....	497,2	.....	92	11,15 a. m..	500,3	.....	96
11,30 a. m....	496,0	.....	92	11,30 a. m..	500,2	.....	96
11,45 a. m....	495,3	.....	92	11,45 a. m..	499,3	.....	92
12 a. m....	494,8	— 1,04	92	12 a. m..	498,9	— 0,22	96
12,15 p. m....	494,0	.....	92	12,15 p. m..	498,4	.....	93
12,30 p. m....	493,9	.....	92	12,30 p. m..	497,9	.....	96
12,45 a. m....	493,8	.....	92	12,45 p. m..	497,3	.....	96
1 p. m....	493,7	—1 ,26	92	1 p. m..	496,9	— 0,62	93
1,15 p. m....	492,9	.....	92	1,15 p. m..	496,9	.....	96
1,30 p. m....	492,4	.....	92	1,30 p. m..	496,7	.....	96
1,45 p. m....	492,2	.....	92	1,45 p m..	496,7	.....	93
2 p. m....	492,0	— 1,60	85	2 p m..	496,5	— 0,70	93
2,15 p. m....	491,7	.....	85	2,15 p m..	496,5	.....	96
2,30 p. m....	491,3	.....	85	2,30 p m..	496,5	.....	96
2,45 p. m....	491,2	.....	85	2,45 p m..	496,5	.....	96
3 p. m....	491.1	— 1,78	85	3 p m..	496,7	— 0,66	96
3,15 p. m....	491,2	.....	85	3,15 p m..	497,1	.....	96
3,30 p. m....	491,2	.....	85	3,30 p. m..	497,1	.....	96
3,45 p. m....	491,2	.....	85	3,45 p. m..	497,1	.....	96
4 p. m....	491,3	— 1,74	85	4 p. m..	497,2	— 0,56	96
4,15 p. m....	491,8	.....	85	4,15 p. m..	497,3	.....	96
4,30 p. m....	491,9	.....	85	4,30 p. m..	497,0	.....	96
4,45 p. m....	492,0	.....	85	4,45 p. m..	497,4	.....	96
5 p. m....	492,0	— 1,60	88	5 p. m..	497,4	— 0,52	96
5,15 p. m....	492,2	.....	88	5,15 p. m..	497,5	.....	96
5,30 p. m....	492,3	.....	88	5,30 p. m..	497,6	.....	96
5,45 p. m....	492,5	.....	88	5,45 p. m..	497,8	.....	91
6 p. m....	492,8	— 1,44	88	6, p. m..	498,0	— 0,40	91

These results are graphically expressed in the diagram of plate XXXII.

DETERMINATION OF CRITICAL MOISTURE POINT FOR MOULDING

Small random samples of cacao beans, both fermented and unfermented (taken from the samples N. 3 and 5), composed of 20 seeds each, were selected. A mixture of conidia and spores of *Aspergillus niger, A. fumigatus, A. glaucus, A. flavius, Rhizopus nigricans, R. arrhizus* and *Mucor mucedo* from fresh carrot agar cultures was prepared and kept dry in a desiccator. Each sample of cacao was enclosed in a common glass cup, and inoculated by dusting (using a small brush) with the mixture of conidia, then covered with a photographic glass plate. Furthermore, in order to regulate the amount of moisture of the atmosphere of the glass cup and to equilibrate the moisture content of the beans with the moisture of enclosed air, a 20 c.c. beaker containing a definite saline solution was inclosed in the glass cup.* The plate glass cover was sealed to the cup with paraffin.

* For references on the method for maintaining constant humidity, see Spencer, H. M. Laboratory methods or maintaining constant humidity.

The following table shows the saline compounds used and the respective concentration of each. As the experiment was made in the Laboratory, and of course, not at a constant temperature, the relative humidity oscileated between two extreme limits. Maximum, minimum and average relative humidity was approximately calculated from Spencer curves or tables.

CONCENTRATION OF THE SOLUTIONS AND RELATIVE HUMIDITY

Salts	Concentration of solution	32 °C. % humidity (aproxim.)	24 °C. % humidity (aproxim.)	28 °C.% humidity (aproxim.)
H_2O Distilled (test)		100	100	100
H_2SO_4 concentrated (test)		Almost 0.	Almost 0.	Almost 0
$Na_2CO_3.10\ H_2O$	Saturated.	81	87	84
$Na_2SO_4.10\ H_2O$	Saturated.	88	92	90
Na Cl and KNO_3	Saturated.	34	30	32
$(NH_4)_2SO_4$	Saturated.	82	88	81
NaOH	N	80	81	80
NaOH	5N	80	81	81
NaOH	10N	84	85	84
NaOH	15N	84	85	85
NH_4Cl	Saturated.	80	79	79
NaCl and $KClO_3$	Saturated.	34	36	35
$KHSO_4$	Saturated.	83	85	84
NaCl, KNO^3 and $NaNO_3$	Saturated.	28	30	29
$BaCl_2.2\ H_2O$	Saturated.	86	88	87
$ZnSO_4.7\ H_2O$	Saturated.	88	90	89
$NaCr_2O7.2\ H_2O$	Saturated.	48	52	50

The experience begun November 13 and ceased February 15 of the subsequent year. The results were as follows:

CACAO MOULDING AS RELATED TO RELATIVE HUMIDITY

First development of moulds	Days from the beginning	Average relative humidity	Solution of
Fermented cacao beans			
November 21	8	90	Na_2SO_4
November 23	10	84	Na_2CO_3
November 23	10	100	H_2O
November 25	12	87	$BaCl_2$
November 26	13	81	$(NH_4)_2SO_4$
November 26	13	85	NaOH. 15N
November 27	14	84	NaOH. 10N
November 28	15	84	$KHSO_4$
November 28	15	89	$ZnSO_4$
November 29	16	81	NaOH. 5N
November 29	16	80	NaOH N
November 29	16	79	NH_4Cl.
Unfermented cacao beans			
November 19	6	100	H^2O
November 20	7	90	Na_2SO_4
November 20	7	89	$ZnSO_4$
November 21	8	85	NaOH. 15N
November 21	8	84	NaOH. 10N
November 21	8	84	$KHSO_4$
November 22	9	87	$BaCl_2$
November 22	9	81	$(NH_4)_2SO_4$
November 23	10	81	NaOH. 5N
November 23	10	80	NaOH. N
November 23	10	79	NH_4Cl
November 24	11	84	Na_2CO_3

DISCUSSION OF THE RESULTS AND CONCLUSIONS

The results of the experiments may be summarized as follow:

(1) The normal moisture content of fermented and unfermented cacao beans varies from 14 to 21 per cent, based on 9 determinations of 9 samples, with a general average of 19 per cent. Moisture content of unfermented cacao beans is higher than the fermented cacao beans, being respectively, 19 and 15 per cent. Also, the moisture content is very variable in different samples.

(2) Also the specific weight varies from a minimum of 0.883 to a maximum of 0.991; this difference is partially related, in our opinion, to the different moisture content. The general average based on 40 determinations on 4 samples is 0.934, but the average of unfermented cacao is slightly higher than in fermented cacao: respectively 0.890 and 0.887.

(3) The size of fermented and unfermented cacao beans is almost the same, the general average being 20 mm. in length, 12 mm. broad and 7 mm. thick.

(4) The capacity of imbibition of cacao beans when immersed in distilled water at 40° C. is about 18 per cent of the initial weight, but varies from 15 per cent (fermented cacao beans) to 20 per cent (unfermented cacao beans). The imbibition is complete during the first 24 hours of immersion.

(5) The capacity of imbibition of cacao beans when exposed to saturated water vapor at room temperature is about 1.10 per cent of the initial weight; these results were obtained in a closed room during five days, at room temperature. Daily absorption is extremely irregular, varying from 2.38 per cent to 0 per cent. The total absorption during the five days is as irregular as the preceding, oscillating from a minimum of 0.56 per cent to a maximum of 2.77 per cent. The average total absorption of unfermented beans (1.57 per cent) is greater than the average of fermented beans (0.56 per cent).

(6) The effect of drying at different temperatures ranging from 40° C. to 100° C. to the absorption of water vapor, when dried cacao beans are exposed to saturated water vapor during ten days, is very clear on fermented cacao beans. The absorption of water vapor is inversely proportional to the temperature of drying, being maximum at the lowest temperature (40° C. and 1.10 per cent of increase in weight) and minimum to the highest temperature (of 80° C., 90°C.,

and 100° C. respectively 0.42, 0.43 and 0.44 per cent). The best temperature for desiccation of fermented cacao beans, as related to the water vapor absorption, is 80° C. On the contrary, the desiccation of unfermented cacao at different temperatures is apparently without influence on the water absorption, the course of increase in weight being very irregular.

(7) The loss of moisture of cacao beans exposed to direct sunshine during 11 hours (7 hours during the first day, and 4 hours during the second) is as irregular as the imbibition of the seeds when exposed to saturated water vapors. In a general way, it is strongest during the first hour, and gradually but irregularly decreased during the following four hours, falling during the 6th and 7th hours. From the 8th to the 11th hour the loss in weight is almost uniform. The general average of loss in weight is 2.03 per cent, but varying from 3.66 per cent to 1.31 per cent. The desiccation is highest in unfermented cacao beans (average 2.27 per cent) than in fermented cacao beans (average 1.78 per cent), according the greater amount of moisture of unfermented seeds as related to fermented seeds.

(8) The average daily weight of samples of fermented and unfermented cacao, recorded during 100 days, is apparently without direct relation to the average daily relative humidity. It is probable that the relation is not simple and exclusive, and other factors influence the variation in weight of cacao beans, as atmospheric pressure, solar irradiation, and so on. These results are confirmed by the record of hourly variation in weight of fermented and unfermented cacao and relative humidity of the atmosphere.

(9) A 24 hours record of samples of cacao beans, both fermented and unfermented, exposed to the free air, taken at the interval of 15 minutes showed a marked variation of increase and loss in weight of fermented or unfermented beans. Moisture absorption of fermented cacao oscillates between 1.10 per cent and 1.78 per cent, with a total range of 2.88 per cent. Moisture absorption of unfermented cacao varies between 2.02 per cent to 0.70 per cent, with a total range of 2.72 per cent. In spite of the fact that the total range of the weight is almost the same in both samples, fermented cacao lost 0.68 per cent in weight, and unfermented cacao increased 1.38 per cent in weight.

The abundant literature on cacao problems, shows that the most important determinations of moisture content of beans and its variations were made in West Africa on unfermented cacao of Accra

type, by Schwarz (**26**). This author found a moisture content varying from 7.16 per cent to 15.32 per cent. Selecting the beans on the base of dryness, he subdivides them into: (1) beans quite pliable in the shell, (2) beans in which the nib was of a cheesy consistency and presented a wet looking surface when cut, and (3) beans which appeared to be fairly dry. Their respective average for three determinations each were: (1) 14.50, (2) 11.33, (3) 8.15. These results agree well with the results obtained on Santo Domingo cacao. The same author performed three experiments on the relationship between humidity and moisture content of cut and shelled cacao beans, weighing a few seeds as well as bags of cocoa. He demonstrated that cut cacao readily absorbed moisture, and that the amount absorbed varied with the humidity. Percentage of moisture varies from 6 per cent to 14.98 per cent. Since the percentage of absorbed moisture agrees with our results, we cannot demonstrate the relationship between moisture of the air and moisture of the cacao. Shelled beans absorbed from 1.32 per cent to 3.96 per cent of moisture. According Schwarz's results, the maximum increase in weight of cacao in burlap bag was only 0.47 per cent. Finally, according the same author, some data obtained from the Gold Coast Department of Agriculture indicated that beans containing in excess of 10 per cent of moisture are prone to mould. From a general point of view, Accra cacao offers a noteworthy analogy with Sánchez cacao, but a most complete picture of moisture in Gold Coast cocoa is desirable for the purpose of comparison.

One of the most important determination is the critical moisture point. The writer ascertained that a relative humidity of 79 per cent permits the development of moulds after 16 days of incubation on fermented cacao, the minimum time required being 8 days at a relative humidity of 90 per cent. On unfermented cacao, the minimum of moisture required is 79 per cent, the incubation during 10 days, and the minimum time 6 days at 100 per cent of relative humidity. The writer does not express the critical moisture point as moisture content of the beans, but surely the moisture is greater than 10 per cent, as indicated in Cold Coast.

These results may be favorably compared with the experiments performed in the Philippines on moulding of copra by Lava (**17**). He found that the critical moisture point at room temperature is 81 per cent, and the period of incubation from 7 to 21 days.

No continuous series of records of relative humidity of Santo

Domingo are available to date, but records for scattered years for Santo Domingo City, Haina, Moca, Santiago, Puerto Plata and Sánchez. The general average is comprised between 65 and 70 per cent, with two minima 60–65 per cent (December to March, and June to August) and two maxima of 70–75 per cent (April–May, and September–November). From 12 to 4 o'clock, during the night, the percentage of relative humidity very rarely drops to 80 per cent, at sea level, and it is surely higher at 600 meter elevation, which is limit of cacao cultivation in Santo Domingo.

The writer concludes that both climatic and metereologic conditions in Santo Domingo, are very frequently favorable to the development of moulds on cacao beans, taking in consideration the commonly very bad preparation of cacao by farmers, as explained in the latter part of this paper.

PREVENTION OF MOULDING OF CACAO

The problem of the prevention of moulding of cacao must be considered under two aspect: (1) the most general and most important, concerning a better preparation of cacao, chiefly from the standpoint of drying, (2) the more specialized, concerning an appropriate storage on farms, villages and depots at the shipping ports, and in steamship holds.

The first aspect of the problem, or the defects in the preparation of cacao, is, more or less, the same in all the larger cacao-countries of the world. In a previous Report (**7**), the writer examined the defects common in Santo Domingo, of which the most important are:

(1) The almost general tendency to produce unfermented cacao which is more susceptible to moulding than the fermented cacao.

(2) The very common practice of mixing cacao beans from healthy and mature pods with beans from diseased, over ripe and immature pods which have the tendency to become moldy.

(3) Buying and selling cacao beans on wet or semidry basis, and in a general way, incomplete or imperfect or inadequate drying of cacao. Many causes co-operate to this tendency, as, e. g., indigence of small farmers, imperfect equipment of farmers, keen competition among brokers, lack of attention in drying, inadequate protection and equipment of brokers, storekeepers, exporters, and others.

(4) Inadequate legislation, inspection and grading before and after storage.

The second aspect of the problem, or the conservation of cacao in stores or depots or steamship holds at a satisfactory percentage of humidity could be examined, if necessary, under the basis of Lava's proposition for prevention of copra moulding, using solid NaCl or solid $NaNO_3$ which maintained, at the temperature of 26–33°C., a relative humidity of about 73 per cent. $CaCl_2$ or CaO must be used if a greater reduction of humidity is desired. The use of these substances is economical, since after the complete or partial solution, they can be easily recovered or, in the case of CaO, used as calcium hydroxide. The problem of high humidity in the holds of the vessels and increase of moulding of cacao beans during the shipment must be studied.

In our opinion, the gradual elimination of the unsatisfactory conditions listed will be the best method to improve the Dominican cacao.

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SUMMARY

Experiments on the environmental conditions influencing the moulding of fermented and unfermented Dominican cacao beans were carried.

IV. TESTS FOR ENZY[illegible] CACAO

The knowledge of the enzymes of c[illegible] beans, both in fresh and dry seeds (fermented and unfermented), is the basis for the understanding of the mechanism and effect of the fermentation. Scattered notices on this subject are found in the literature of cacao technology, but the first complete study was performed in the Philippine Island by Brill (1). This investigation has been undertaken for a comparison with Brill's results, a few of which are different from the results obtained by previous investigators, as well as for testing enzymes not previously considered.

METHOD OF STUDY

Four series of experiments were carried out, using unselected

Dominican cacao (Sánchez type), harvested in Moca and prepared in the National Agronomic Station, namely: (1) fresh cacao beans with surrounding pulp, (2) fresh and clean cacao beans without the pulp, (3) clean fermented cacao beans, prepared by fermenting the seeds during four days at the temperature of the laboratory (20–30°.C) and then drying, (4) clean unfermented cacao beans, prepared by drying the seeds after carefully washing.

The method used was almost the same as the method employed by Brill: a 10 per cent aqueous solution of ground seeds, standing six hours and then filtered through cloth, was mixed with 2 per cent (by volume) of toluene and 2 per cent of chloroform, both as antiseptic and as facilitating the exosmose of the enzymes.

Each experiment was repeated at least three times, and more, if necessary, until a confirmation of positive or negative results was obtained.

Saccharase. 10 c.c. of water, 10 c.c. of cacao bean extract, 10 c.c. of 40 per cent cane-sugar solution, 3 drops of very diluted chloridric acid (pH of the mixture 4.6), standing 48 hours at 40° C., and testing with Fehling solution.

(1) Fresh beans and slime: strongly positive.
(2) Fresh clean beans: positive.
(3) Fermented beans: negative.
(4) Unfermented beans: slightly positive.
(5) Test: negative.

Maltase. As in the preceding, but using 10 c.c. of 20 per cent maltose solution (pH of the mixture acidized: 4.2).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

As in the preceding, but using bean extract obtained by 10 days of maceration in thermostate at 40°C. (pH of the mixture, acidized: 4.0).

(1) Fresh beans and slime: slightly positive.
(2) Fresh clean beans: positive.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Lactase. As in the preceding, using a 20 per cent lactose solution (pH of the mixture, acidized: 4.4).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

As in the receding, but after 10 days of maceration (see above); (pH of the mixture, acidized: 5.0).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Trehalase. As in the preceding (pH of the mixture, acidized: 4.4).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Melibiase. As in the preceding (pH of the mixture acidized: 4.8).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Amylase. 40 c.c. of soluble starch solution (1 per cent), 10 c.c. of bean extract, standing 48 hours at 40°C., testing the presence of reducing sugars with Fehling solution.

(1) Fresh beans and slime: strongly positive.
(2) Fresh clean beans: slightly positive.
(3) Fermented beans: negative.
(4) Unfermented beans: slightly positive.
(5) Test: negative.

Dextrinase. As in the preceding, but testing the presence of dextrines with Lugol solution.

(1) Fresh beans and slime: yellowish.
(2) Fresh clean beans: reddish.
(3) Fermented beans: violet-bluish.
(4) Unfermented beans: reddish.
(5) Test: violet-bluish.

Pectinase. 25 c.c. of bean extract, 5 gm. of pectine, freshly prepared from carrots (Bertrand and Mallevre method, as referred by Calmette, Negre and Bouquet: Man. techn. de Microbiol. et Sérol., II édit., p. 168. Paris, 1926), testing the presence of reducing sugars with Fehling solution, after 48 hours at 40°C.

(1) Fresh beans and slime: negative.
(2) Fresh clean béans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Cellulase. 25 c.c. of bean extract, 5 gm. of stripes of ashless filter paper. After 48 hours of incubation at 40° C., the paper is washed, dried and weighed.

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Emulsin. 10 c.c. of bean extract, 10 c.c. of amygdalin (2 per cent solution), standing 48 hours at 40°C., and testing the presence of cyanhidric gas with piero-sodic paper in a corked Erlenmeyer.

(1) Fresh beans and slime: pinkish.
(2) Fresh clean beans: pinkish.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

As above, but testing with 10 drops of freshly prepared Fe SO_4 solution (3 per cent), one drop of Fe Cl_3 (1 per cent), mixing thoroughly, adding NaOH solution (10 per cent) and dissolving the precipitate with H_2SO_4 solution (10 per cent).

(1) Fresh beans and slime: light Prussian-blue color.
(2) Fresh clean beans: slight Prussian-blue color.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Protease. 5 gm. of coagulated egg albumine, 25 c.c. of bean extract, for 10 hours at 40°C., testing the loss in weight of albumine cylinders (Mett method, Arch. Anat. und Physiol., Physiol. Abth., *68:* 94). Liquid media acidified with a few drops of very diluted chloridric acid (pH 3.8).

(1) Fresh beans and slime: negative (loss gm. 0,003).
(2) Fresh clean beans: negative (loss gm. 0,008).
(3) Fermented beans: negative (loss gm. 0,004).
(4) Unfermented beans: negative (loss gm. 0,008).
(5) Test: negative (loss gm. 0,005).

Tryptase. One cubic centimeter of 30 per cent solution of gelatine in thymol solution (0,1 per cent) is distributed in each test tube of 5 mm. in diameter, being previously alcalinized with 2 per cent of NaOh N/10, adding 1 c.c. of bean extract to each test tube. After 48 hours at 30°C., the height of the cylinder of gelatine is measured (Fermi method, as referred to De Rossi, Microbic. agr. e teen. 204.1927).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Peptase. 10 c.c. of egg albumen solution (one egg albumen in 100 c.c. of water), 10 c.c. of bean extract, for 48 hours at 40°C (pH of the solution:

6.2). Acidity of dissociated amynoacid is titrated with KOH 1/10 N(Sörensen's tritimetric method, as referred in De Rossi, 1. c., p. 205).

(1) Fresh beans with slime: c.c. 1, 0.
(2) Fresh clean beans: c.c. 0,9.
(3) Fermented beans: c.c. 0,6.
(4) Unfermented beans: c.c. 0,8.
(5) Test: c.c. 0,6.

20 c.c. of egg albumen solution, prepared as above, 10 c.c. of seed extract, standing 48 hours at 40°C. Indole test performed by Goré modification of the technic of Ehrlich-Böhme (as referred by the Comm. on Bact. Techn., Soc. Amer. Bacteriol. Manual meth. pure culture study of Bacteria, p. VI/14. 1930).

(1) Fresh beans with slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

20 c.c. of Bactopeptone solution (2 per cent), 10 c.c. of bean extract, for 48 hours at 40°C. Indole tested as above (pH=6, 2).

(1) Free beans with slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Amynoacidase. 20 c.c. of asparagine (2 per cent solution), 10 c.c. of been extract, for 48 hours at 40° C., testing the alcalinity with KOH N/10.

(1) Fresh beans with slime: c.c. 2, 2.
(2) Fresh cleans beans: c.c. 2, 3.
(3) Fermented beans: c.c. 1, 6.
(4) Unfermented beans: c.c. 2, 0.
(5) Test: c.c. 1, 5.

Lipase. 10 c.c. of water, 10 c.c. of bean extract, 1 c.c. on pure olive oil, 1 drop of diluted acetic acid, for 48 hours at 40°C., testing the acidity with NaOH N/10.

(1) Fresh beans with slime: c.c. 2, 2.
(2) Fresh clean beans: c.c. 1, 7.
(3) Fermented beans: c.c. 2, 91.
(4) Unfermented beans: 5, 9.
(5) Test: c.c. 2, 1.

Glycerophosphatase. 10 c.c. of water, 10 c.c of bean extract, 50 c.c. of 2 per cent solution of sodic glycerophosphate, 48 hours at 40° C. (pH = 5, 8), testing the presence of soluble phosphoric acid with ammonium molybdate solution.

(1) Fresh beans with slime: slight yellow coloration.
(2) Fresh clean beans: slight yellow coloration.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Fitase. 20 c.c. of bean extract, 30 c.c. of 2 per cent solution of fitine, 48 hours at 40°C. (pH=6.2), testing the presence of soluble phosphoric acid with ammonium molybdate solution.

(1) Fresh beans with slime: slight yellow coloration.
(2) Fresh clean beans: slight yellow coloration.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Oxidase and peroxidase 20 c.c. of seed extract, 1 c.c. of alcoholic tincture of guaiacum (10 per cent), the color being observed after ½ hour, 2 hours, 12 hours and 24 hours of thermostation at 40°C.

(1) Fresh beans with slime: light red; red; dull read; dull red.
(2) Fresh clean beans: pinkish; red-pinkish; red; red.
(3) Fermented beans: no change; pinkish; red; pinkish.
(5) Unfermented beans: pinkish; red-pinkish; red; red.
(5) Test: no change.

5 c.c. of bean extract, 1 c.c. of benzidine solution (0.5 per cent) and 5 drops of H_2O_2, 2 hours at 40°C.

(1) Fresh beans with slime: violet-bluish.
(2) Fresh clean beans: light violet-blue.
(3) Fermented beans: very light violet-blue.
(4) Unfermented beans: violet-blue.
(5) Test: unchanged.

5 c.c. of bean extract, 1 c.c. of solution of acid pyrogallic (2 per cent), 5 drops of H_2O_2, after 24 hours at 40°C.

(1) Fresh beans with slime: duty yellow with precipitate.
(2) Fresh clean beans: dirty yellow with precipitate.
(3) Fermented beans: very light yellow.
(4) Unfermented beans: dirty yellow.
(5) Test: unchanged.

Catalase. 5 c.c. of bean extract 50 c.c. of neutralized H_2O_2 (pH=6.8.) 1 per cent solution, measuring the O developed, using a common calcimeter, after 24 hours at the temperature of Laboratory (24–30°C.).

(1) Fresh beans with slime: 31, 1 c.c. of oxigen.
(2) Fresh clean beans: 29, 2 c.c. of oxigen.
(3) Fermented beans: 2, 7 c.c. of oxigen.
(4) Unfermented beans: 11, 8 c.c. of oxigen.
(5) Test: 0, 8 c.c. of oxigen.

Philotion. 10 c.c. of bean extract, 2 gm. of precipitated sulphur contained in closed Erlenmeyer flask in presence of stripes of lead acetate paper, after 24 hours at 40°C.

(1) Fresh beans with slime: very light brown color.
(2) Fresh clean beans: very light brown color.
(3) Fermented beans: no change.
(4) Unfermented beans: very light brown color.
(5) Test: no change.

Reductase. 10 c.c. of bean extract, 10 c.c. of water, 1 c.c. of methylene blue solution at 1 per cent, after 48 hours at 40°C.

(1) Fresh beans with slime: slightly decolorized.
(2) Fresh clean beans: no change.
(3) Fermented beans: no change.
(4) Unfermented beans: no change.
(5) Test: no change.

DISCUSSION OF THE RESULTS

The results of our experiences, as compared with the tests performed by Brill, may be summarized as follows:

Enzyme (1)	Brill's experiments					Author experiments				
	Pulp	Heated pulp	Fresh clean seeds	Heated fresh clean seeds	Fermented seeds	Heated fermented seeds	Fresh seeds with slime	Fresh clean seeds	Fermented seeds	Unfermented seeds
A-HYDROLYZING ENZYMES										
a-DECOMPOSING CARBOHYDRATES										
1-Saccharase (invertase)	P	P	N	N	P	P	P	P	N	P
2-Maltase	N	N	N	N	N	N	P	P	N	N
3-Lactase							N	N	N	N
4-Trehase							N	N	N	N
5-Melibiase							N	N	N	N
6-Raffinase	P	P	P	P	P	P				
7-Amylase (diastase) (2)	N	N	N	N	P	P	P	P	N	P
8-Dextrinase (3)	N	N	N	N	?	N	P	N	N	N
9-Inulase	N	N	N	N	N	N				
10-Pectinase							N	N	N	N
11-Cellulase							N	N	N	N
b-DECOMPOSING GLUCOSIDES										
1-Emulsin	N	N	N	N	N	N	P	P	N	?
c-DECOMPOSING PROTEIC SUBST.										
1-Protease							N	N	N	N
2-Tryptase							N	N	N	N
3-Peptase (indole test)							N	N	N	N
4-Albuminase (tryptophane test)	?	N	N	N	N	N				
5-Protease (tryptophane test)	P	N	N	N	N	N				
6-Amynoacidase							?	?	N	?
d-DECOMPOSING FAT SUBSTANCES										
1-Lipase	N	N	N	N	N	N	N	N	N	P
e-DECOMPOSING ETHER. COMP. PHOSPHORIC ACID										
1-Glycerophosphatase							P	P	N	N
2-Fitase							P	P	N	N
B-OXIDING AND REDUCING ENZYMES										
1-Oxidases	P	N	P	N	P	N	P	P	P	P
2-Peroxidases							P	P	P/?	P
3-Catalase							P	P	?	P
4-Philothion							P	P	N	P
5-Reductase	P	P	P	P	P	P	P	N	N	N

(1)—N-Negative results; P-Positive results; ?-Doubtful results.
(2)—The name of enzymes enclosed in the brackets, are used in the Brill's paper.
(3)—In the study of Brill, the dextrinase is comprised in the test for diastase.

The hydrolyzation of the saccharose is a controversial point. According Brill (1), fresh clean cacao beans do not hydrolyze this sugar, while, in our experience, the enzyme is found on seeds with slime, clean seeds and dried cacao beans, but not in the fermented cacao. Different results can be explained by the different environmental conditions of the tests, such as concentration of the sugar solution, hydrogen-ions concentration, etc.

The tests for maltase in the Philippine cacao were negative, and Brill affirmed that "this appears to be one of the rarer cases of diastases unaccompanied by maltase". Our first test, repeating, more or less, the conditions of the tests performed by Brill showed negative results. A second series of tests, varying the duration of

the incubation (ten days instead of two days), showed positive results only in fermented and unfermented cacao beans. It is well known that the maltase is an endoenzyme not easily extracted from the plant cell, and only from dried material, after a long maceration.

Amylase (an enzyme hydrolyzing starch to dextrines) is not present in fermented beans only, but dextrinase (an enzyme hydrolyzing dextrines to maltose) was in beans without slime, fermented and unfermented cacao. These results are in contrast with Brill's results and are confusing.

In our experiment, the presence of amygdaline was demonstrated, at least in fresh seeds (with and without slime), in accordance with the results of Sack (cfr. H. Smith. Ferment of cacao, p. 148. London 1913), but in disagreement with Brill's results.

Of the enzymes hydrolyzing proteic substances, or its derivates, the presence of the amynoacidase is doubtful; hydrolizing enzymes, both in acid and alcaline media, are absent. These results are in partial disagreement with Brill's tests.

Lipase is present only in dry unfermented seeds. Glicerophosphatase and fitase are secreted by fresh seeds.

Oxiding and peroxiding enzymes are contained in fresh and dry cacao beans, as well as, in part, the enzyme transforming sulphur in sulfuretted hydrogen. Only fresh seeds with slime contain reductase. Brill found reductase in pulp, fresh, and fermented seed, also heated.

CONCLUSIONS

From the results of these investigations, definite conclusions cannot be drawn without a deep knowledge of organic compounds contained in slime, fresh fermented and unfermented cacao beans, as well as without the study of enzymatic activities of cacao-fermenting organisms. Unfortunately, these observations are fragmentary and rather incomplete.

Of twenty-two tested enzymes, only oxiding and peroxiding enzymes are universally diffused on different kinds of cacao beans. Fresh seeds with slime are of the amplest range of enzymatic activity (13 enzymes); fresh clean seeds (without slime) contain 11 enzymes, tests for dextrinase and reductase being negative. Fermented dry seeds contain 9 enzymes, the range of the enzymatic activities being limited to the invertase, diastase, lipase, oxiding and peroxiding enzymes, including the doubtful presence of the amynoacidase and emulsin. Positive test for the presence of a

lipolytic enzyme, (splitting fatty substances into glycerol and fatty acids) ascertained only in unfermented seeds should be particularly mentioned, as related with the rank odor of some Dominican unfermented samples of cacao. The presence of the lipase must be confirmed testing fatty substances composed of higher fatty acids as sterinolipoids, recently obtained on cacao beans. In spite of the fact that the presence of a relatively large amount of fats in cacao beans indirectly confirms the necessary presence of a lipolytic enzyme, this test is not very sure for the presence of the glicerophosphatase and fitase. If glicerophosphatids and fitine are present in cacao seeds, the acidity of the test on fatty substances should be derivated, at least in part, from the hydrolyse of esters of phosphoric acid.

Fermented cacao lacks almost totally of enzymatical activities; only three enzymes were obtained, of which two are of doubtful presence. The only enzyme surely present is the oxidase, probably associated with the frequent blackening of old Dominican fermented and unfermented cacao. The poor enzymatic activity (from qualitative standpoint) of fermented cacao, as probably related with a greater stability of the seeds, could be favorably compared with the more active dried unfermented seeds. The fermented cacao bean is a truly dead seed, also from biochemical point of view, while an unfermented cacao bean is apt, under suitable conditions, to many biochemical changes resulting from its latent enzymatic activity.

SUMMARY

Twenty-two enzymes are tested on Dominican cacao. Fresh cacao beans with surrounding slime are the most active; fermented dry cacao beans are almost inactive. A lipase is probably present only in fermented cacao.

V. AN IMPROVED METHOD FOR THE DESICCATION OF CACAO BEANS

This method is based on the application of a distillation box for saline water, when exposed to the sunshine, for desiccation of fermented or unfermented cacao beans.

The principle of this apparatus, attributed to Charles Wilson, is well known: the saline water is contained in a metallic dish, enclosed in a flat wooden box; the top if formed by an inclined glass plate, and exposed to the direct sunshine. To each descent of temperature, the saturated water vapor enclosed in the box is condensed on the glass plate, and is collected in a bottle as distilled water.

This distilling box was used for the first time during the year 1872 for securing the supply of drinking water for the workers employed in the silver mines of Salinas, in the desert of Atacama (Chile), from a saline water containing 140 gr. of salt per liter. Using a series of Wilson's apparatus, with a glass surface of about 5,000 square meters, the production of distilled water was 23 metric tons per day.

The same apparatus was employed for many other uses, by Maurain and Brazier in Paris, Ginestous in Tunisy, Richard in Monac, Lozano in Spain, and others for the industrial drying of fish, fruit, distillation of alcohol, etc. This method and its application was described in detail by Richard (**24**) and by Lozano (**21**).

During the summer of 1929 I constructed an apparatus of about one square meter of glass surface, and during the months of August and September I experimented on the possibility of the using it in cacao bean drying. As these preliminary observations, under certain conditions, gave good results, I delayed the study of the practical application using a larger series of boxes to the summer of 1930, when the National Agronomic Station of Moca was closed.* For this reason the observation here referred to must be considered only as preliminary results, and the publication is made for the purpose of stimulating experiments on an industrial scale.

The structure of Wilson apparatus is explained by the sectional sketch of pl. XXXIII: ** *di* is the metallic dish containing the cacao beans; *wa* are the wooden walls; *pl* the glass plate; *mp* is a metal plate bended to Z shape, for the detention of drops; *co* is a gutter functioning as a water collector; *pi* indicate a small pipe line for conduction of the water to the bottle *bo* and four supports *fo*. One small door, *do* opening at the right side makes it possible to remove the metallis dish. The box is black painted and the joints (chiefly the glass plate joins) are sealed with sealing wax.

During many weeks, the temperature was recorded using a double-recording thermograph (air and soil thermograph) for simultaneous record of inside and outside temperature. In spite of the fact that the registrating pen for the box temperature was set so that the 0°C. line of the chart corresponded to 30°C., with a few

* A few experience on drying of fresh fruit, also made favorable results, using oranges, pawpaw, cashew fruits, *sapote* and *mamey*, but not completely good using star-apple, plantain and banana, also if cutted in small pieces.

** The figure is taken from Lozano paper.

exceptions, the maximum temperature was not included in the graphic. In other words, in a general ways, the maximum temperature is greater than 75°C., while the maximum is, more or less, the same as the free air, or slightly less. As exemplification, the two-hour temperature of the box and the temperature of the air is reported, from Wednesday September 25 to Sunday September 29; the graphic is illustrated in the plate XXXIV.

Day	Hour	Air temperature degrees C.	Averages degrees C.	Box temperature degrees C. (1)	Averages degrees C.
September 25	2 a. m.	24		24	
September 25	4 a. m.	23		23	
September 25	6 a. m.	22		35	
September 25	8 a. m.	21		55	
September 25	10 a. m.	28		75	
September 25	12 a. m.	31	25, 0	65	46, 0
September 25	2 p. m.	36		45	
September 25	4 p. m.	41		29	
September 25	6 p. m.	29		26	
September 25	8 p. m.	25		25	
September 25	10 p. m.	23		24	
September 25	12 p. m.	22	29, 0	23	29, 0
September 26	2 a. m.	22		22	
September 26	4 a. m.	21		21	
September 26	6 a. m.	21		38	
September 26	8 a. m.	23		55	
September 26	10 a. m.	28		80	
September 26	12 a. m.	32	24, 5	74	48, 0
September 26	2 p. m.	40		40	
September 26	4 p. m.	39		27	
September 26	6 p. m.	28		26	
September 26	8 p. m.	24		24	
September 26	10 p. m.	24		23	
September 26	12 a. m.	23	29, 5	22	27, 0
September 27	2 a. m.	21		23	
September 27	4 a. m.	21		24	
September 27	6 a. m.	23		27	
September 27	8 a. m.	23		37	
September 27	10 p. m.	24		22	
September 27	12 a. m.	29	23, 5	57	39, 0
September 27	2 p. m.	21		43	
September 27	4 p. m.	38		27	
September 27	6 p. m.	29		25	
September 27	8 p. m.	25		25	
September 27	10 p. m.	24		25	
September 27	12 p. m.	24	27, 0	25	26, 0
September 28	2 a. m.	24		25	
September 28	4 a. m.	24		24	
September 28	6 a. m.	23		37	
September 28	8 a. m.	24		58	
September 28	10 a. m.	28		77	
September 28	12 a. m.	31	26, 0	70	48, 0
September 28	2 p. m.	38		43	
September 28	4 p. m.	41		28	
September 28	6 p.m.	28		27	
September 28	8 p. m.	26		25	
September 28	10 p. m.	24		24	
September 28	12 p. m.	23	30, 0	24	28, 5
September 29	2 a. m.	23		25	
September 29	4 a. m.	24		28	
September 29	6 a. m.	24		40	
September 29	8 a. m.	27		55	
September 29	10 a. m.	29		77	
September 29	12 a. m.	32	26, 5	70	48, 5
September 29	2 p. m.	40		38	
September 29	4 p. m.	41		38	
September 29	6 p. m.	29		27	
September 29	8 p. m.	26		27	
September 29	10 p. m.	26		26	
September 29	12 p. m.	26	31	25	28, 5

(1) The figures corresponding to the curves outside of the chart are approximately calculated.

It should be noted that (1) these days were exceptionally warm, (2) the depression of the temperature of the box at about 4 o'clock corresponds to the shading of the same caused by the respective positions of the Wilson apparatus and the walls of the laboratory house, (3) that not only the Wilson's box and the sensitive thermometric cylinder, but also the air temperature registrating element and the body of thermograph were exposed to the full sunshine, on a reflective cement floor, (4) the great thermometric depression from 10 to 12 o'clock of the day September 27 corresponds to a drizzling rain.

A series of records obtained using a maximum and minimum alcohol thermometer showed that the maximum daily temperature exists from 2 to 4 o'clock in the afternoon, and oscillates between 65 and 83°C. when the day is warm and the sky is bright, and from 35 to 50°C. during rainy or dull days. Minimum temperatures correspond to the minimum of free air.

The condensation of water vapor reaches the maximum from 4 P.M. to 6 P.M. and about three fourths of the condensed water is collected during these two hours. Also a depression of the temperature during the maximum elevation is very productive in condensed water. From 4 to 10 A.M. the collection of condensed water is only occasional; no water is collected during the night.

During the first experiment, a layer about 12 cm. in thickness (5 inches) was placed in the metallic dish of the distilling box, the condensation being abundant, but the desiccation not uniform, so that frequent stirring was necessary. The best thickness of the layer of cacao beans is not more than 5 cm. (about 2 inches). Under the best conditions, one to three days are necessary for a complete drying of the seeds, and generally two days, when the cacao is unfermented, and from two to four days (most frequently three days) when unfermented. If the beans are over-dried, they appear slightly wrinkled and friable, but of good flavor and odor.

Two or three consecutive rainy days are very dangerous, if fermented or unfermented cacao beans are freshly stored in the distillation box, as the atmosphere saturated with water vapor favors the continuation of fermentation, and of course, an over-fermentation, or the rotting of cacao beans. In this case, the best prevention is the removal of the cacao from the box, and the storage in a room to protect from the rain. One day of rain followed by one day of full sunshine is not so dangerous, as the beans are par-

tially dried, and the raising of the temperature acts as a partial sterilizer.

The distilled liquor is apparently strongly acid when the beans are fermented; and weakly acid if they are unfermented and have the peculiar odor of cacao fermentation, but they were not examined chemically.

In conclusion, the drying of cacao beans by the utilization of the sunshine in such a box is promising for the cacao industry, as an easy, inexpensive and quick method, but more complete studies, made on industrial bases are necessary before forming a definite judgment.

SUMMARY

The application of Wilson's box of water distillation, utilizing the sunshine, for the desiccation of cacao beans is suggested. This method should be studied more thoroughly.

VI. THE YEASTS OF THE DOMINICAN CACAO

Fragmentary notices on the microorganisms, and particularly the yeasts (including asporigenous forms) found on cacao beans or isolated from fermenting cacao, are easily found in many papers on cacao problems, chiefly on curing of cacao. Our knowledge of yeasts of the cacao beans, during the period before the year 1927, is very incomplete. They are summarized in a comprehensive treatise by Hamel Smith et al. (**14**) on cacao fermentation. The only named and apparently specific yeast of the cacao fermentation is the "*Saccharomyces theobromae*" Preyer, but, according to Hamel Smith (**14**), De Rossi (**10**), Henneberg (**15**), and others, the presence and activity of cosmopolite yeasts (such as the elliptic yeast, the apiculate yeast, the anomalus yeast) was commonly admitted. In 1927 Lilienfeld-Toal (**19**) published a very important and exhaustive paper on yeasts (sporigenous and asporigenous), in the German language, based on material from Ecuador, Venezuela, Trinidad, Brazil, Gold Coast, St. Thomé, Ceylon, Java, etc., which was soon followed by another paper by Busse, Henneberg and Zeller (**1** bis) referring to the results of experiments on cacao fermentation.

The microorganisms isolated and studied by Lilienfeld-Toal were: (1) *Saccharomyces ellipsoideus* var. *tropicus* Lil.-Toal & Henneb., (2) *Schizosaccharomyces Bussei* Lil.-Toal & Henneb., (3) the "anomalus" yeast, (4) an undetermined "Kamhefe A", probably identic

with *Saccharomyces theobromae*, (5) an undetermined sporogenous yeast from Brazilian cacao, listed as "Weinhefe B", (6) an undetermined "Kahmhefe B"; isolated from Costa Rican and Trinidad beans, (7) an undetermined "Hefe R", asporogenous, isolated from St. Thomé samples and (8) an undetermined "Saccharomyces M", isolated from Trinidad cacao beans.

Samples of the Dominican cacao (so called 'Sánchez' type) was not included in the study of Lilienfeld-Toal. So far as the present writer is able to learn, there exists no record of experiments on Sánchez cacao, with the exception of an anascosporic yeast (*Kloecheria domingensis* Cif.), isolated from rotting cacao pods by the writer (**5**). As independently from the "vexata quaestio" of the industrial importance of the cacao fermentation, the exact nature of the yeasts causing or associated with fermentation, remains one of the major questions, and constitutes the bases for later studies. The most important purpose of this paper is to characterize the yeasts and pseudo-yeasts found on fermenting Dominican cacao beans.

The study of Lilienfeld-Toal was performed on dry cacao beans, and the yeasts isolated were those most likely adapted to survive during curing and drying. A second and minor purpose of this study was the comparison between the yeasts isolated during the fermentation and the yeasts found on dry fermented cacao beans, and their respective numerical distributions.

MATERIAL AND METHOD

During the years 1926, 1927 and 1928, isolations were made from fermenting cacao in fermetation boxes of cacao farmers located at Samaná, Sánchez, Villa Rivas, Pimentel, San Francisco de Macorís, Salcedo, Moca, Santiago, Bajabonico, La Vega, Bonao and San Cristóbal, with a total of 162 isolations. The determination of the yeasts and the distribution during the fermentation, was performed in the Agronomic National Station at Moca. The study of the yeasts remaining on dried cacao beans was made in combination with the experiments on moulds and mouldings of cacao beans.

As soon as the isolated strain was purified, the fermentation was tested, using Lindner's method for small fermentation and the strain stored. According to Redaelli and the writer's procedure (**22**), the optimum temperature for the growth was observed on the "starting medium" pepto-glucose agar, at $pH = 7{,}00 \pm 0{,}4$), and the sporogenicity on Gorodkowa's agar. The macroscopical and microscopical

morphology was deduced from the study of cultures on (1) Raulin neutral solution, (2) carrot agar, (3) malt extract gelatine (10 per cent solution in surrogation of the beer worth), (4) malt extract water (10 per cent solution). Biochemical activities were tested, in advance of the fermentation tests, the assimilation of the carbon from the carbohydrates, alcohols, and organic acids, and assimilation of azote from organic and inorganic nitrogenous compounds. For a more detailed description of methods of study, we refer to Redaelli and Ciferri (**22**).

The classification adopted by Redaelli and the writer (**9**) as well as by the writer alone (**6**) is followed.

Strains N. 151, 168, 169, 174, 183, 189, 203, 207, 211, 221, 230, 249 and 250

CULTURAL CHARACTERISTICS

Optimum temperature of growth: about 40°C.

Starting medium: colony abundant, of rapid growth, yellowish to yellow, creamy, uniform; edges thinner than the center; border irregularly sinuate.

Gorodkowa's agar: scanty growth. Easily forming spores.

Raulin's neutral solution: poor and slow growth; after two weeks, the solution is troubled; no velum; fragments of the ring; deposit not abundant, almost mucilaginous.

Carrot agar: very abundant growth: not possible to distinguish the colony from that on starting medium; a colony one week old is partly collected at the bottom of the tube.

Malt extract gelatine (geant colony): flattened, whitish-yellowish, more or less round, irregularly bordered colony, without characteristic features; center crateriform, with not well marked radial striae; no appreciable liquefaction. Must be referred to the fundamental type I of Will.

Malt extract water: quick formation of one at first pulverulent, then creamy deposit; formation of ring slow and irregular, frequently also incomplete; no superficial pellicle. A good etheric-alcoholic odor.

The color is a somewhat variable characteristic: the colonies of the strains N. 168, 183, 211 and 230 are yellow in color; strains N. 189 and 211 are almost white; other strains are yellowish to white-creamy.

Also the presence of a depressed crater is a rather variable character, well defined in the strains N. 174, 207 and 221, almost absent in the strains N. 168 and 169. Radial striae are, in a similar way, a more or less variable character.

MORPHOLOGICAL CHARACTERISTICS

Spheric, smooth, 2–4 mmm. diameter ascospores, 1 to 4 for each cell (most frequently 3), formed without previous copulation. Sporification of the cells variable from about 75 per cent (strain N. 183) to about 20 per cent (strain N. 168). Vegetative cells normally spheric, rarely sub-spheric to sub-elliptic, 3–4.5 mmm. in diameter; geant cells scarce, 5–7 mmm. in diameter. Very active, normally unipolar budding. Cells normally single, rarely from 2 to

5-chained (in the strains. N. 174 the cells are generally aggregated in little chains of 3 to 4 elements). No special morphological features. Cells of the deposit on liquid cultures are similar to the vegetative cells on solid media, but sub-elliptic in shape, 2.5–4.5 mmm. by 3–5 mmm. Cells of the ring, as rule, forming small chains (of 2 to 4 elements), showing polar or lateral budding.

BIOCHEMICAL CHARACTERISTICS

Gas production in glucose, saccharose, levulose, raffinose and maltose; fails to produce gas in galactose, mannose, rhamnose, lactose, trehalose, arabinose, xylose, sorbitol, dulcitol, melibiose, starch, soluble, destrin and inulin. It inverts saccharose and raffinose but not trehalose. Assimilates readily peptone, but no nitrate and nitrite of potassium, and ammonium sulphate. Glucose and saccharose are readily taken up; glycerin is very scantly assimilated; acetic and tartaric acids are not assimilated, or very slightly. It fails to liquefy gelatine, although it forms the nail.

SYSTEMATIC POSITION

This always present and abundant yeast belongs to the *Saccharomyces ellipsoideus* var. *tropicus* Lil.-Toal & Henneb., an inter-tropical yeast isolated from fermented beans of many tropical countries. This variety, in my opinion, is no other than the common ellipsoideus yeast of the wine of the temperate countries, adapted to the tropical regions. Differences between our strains and the description of Lilienfeld-Toal are slight except for the optimum temperature of growth (in our strains about 40°C., and in the Lilienfeld-Toal strains from 20 to 30°C., the higher limit being 42°C.), and the copulation as a residual sexuality ascertained by the German author. The optimum temperature of the Dominican strains are apparently more closely related to the temperature of the fermentation boxes, normally oscillating from 35 to 45°C.

STRAINS N. 172, 179, 205, 225, 236, 247, 256 AND 268

CULTURAL, MORPHOLOGIC AND BIOCHEMICAL CHARACTERISTICS

Not possible to distinguish from the preceding yeast, except by the absence of the fermenting power of galactose.

SYSTEMATIC POSITION

This yeast, almost as frequent as the *Saccharomyces ellipsoideus* var. *tropicus*, is of the same cycle, and may be distinguished from the type in a new variety: *Saccharomyces ellipsoideus* var. *domingensis* Cif., n. var. As a matter of fact, this variety is, more or less, of the same importance as the Lilienfeld-Toal's "Weinhefe B", found on Brazilian cacao beans, and a separated from the type chiefly

by the fermentation of glucose and gelactose, not of saccharose, maltose and raffinose, as well as by the tuberculated surface of the colonies on agar media. In my opinion, the brazilian strain may be classed in a variety of the same yeast; *Saccharomyces ellipsoideus* var. *brasiliensis* Cif., n. nom.

Strains N. 155, 159, 173, 186, 196, 223, 244 and 258

All these strains were classified on the basis of morphological characteristics as *Endomyces anomalus* (Hans.) Zender, the cosmopolite yeast formerly known as *Saccharomyces anomalus* or *Willia anomala*.

This yeast is exceedingly frequent, and was found by Lilienfeld-Toal on samples of cacao. Judging from the cultural features on starting medium, one may distinguish many forms or varieties, chiefly by the color of the colonies, varying from the whitish to the pinkish, this characteristic probably being related to other distinctive features, but the study of our strains was limited to the micro-morphologic ones.

Strains N. 154, 166, 175, 190, 201, 224, 237 and 255

Of these strains, N. 166, 190 and 237 only were studied in detail; the identification of the remaining was made from micro-morphologic characteristics. All yeasts here listed must be classified as *Schizosaccharomyces Bussei* Lil.-Toal & Henneb., a cosmopolite yeast found by Lilienfeld-Toal as one of the most diffused. The differences between the description and our strains are not appreciable: among the cultural characteristics, the optimum temperature of growth is 40°C. (37°C. according the description); the color of the colonies varies from light yellow and straw-yellow. The concentric rings of the geant colony are more or less marked, as well as the radial striae. It fails to liquefy the gelose. Assimilates peptone easily, glycocol and asparagine not at all or very scantily; nitrate and nitrate of potassium, and ammonium sulphate not at all. It readily inverts saccharose, raffinose and trehalose. It does not seem to assimilate organic acids, (malic, tartaric, acetic and citric) and glycerine (plate XXXV, fig. C).

As rightly expressed by Lilienfeld-Toal, this species is of the cycle of *Schizosaccharomyces Pombe* Lindner, from which it differs as *Schizosaccharomyces mellacei* Jorg. differs from *S. Pombe*.

For the characteristics of the asporogenous race, see *Schizotorulopsis Bussei.*

Strains N. 152, 165, 185, 206, 219, 220, 235 and 240

The complete study of this yeast was made up on the strains N. 152 and 220 only, and the remaining strains classified according the microscopical appearance of the vegetative cells.

CULTURAL CHARACTERISTICS

Optimum temperature of growth: about 35°C.

Starting medium: colony abundant, thick, of very rapid growth, smooth, uniform; border unbroken, or forming very irregular and broad sinuosities, of enameled polish; ivory-white in color and creamy when young, it appears slightly yellowish and pastose when old.

Gorodkowa's agar: poorly developed. Not forming spores.

Raulin's neutral solution: it forms a heavy creamy ring, at first incomplete, later complete; there is no pellicle; the liquid medium remains clear; deposit mucilaginous, very abundant, roping, grayish in color.

Carrot agar: very similar to the starting medium, but the colonies are more developed and of more rapid growth.

Malt extract gelatine (geant colony): flat, smooth, ivory-white, of small size colony, of enameled polish, with uniform irregularly undulate borders, without crateriform central cavity, radial striae or concentric rings. Growth according the fundamental type I of Will.

Malt extract water: very similar to the growth on Raulin neutral solution.

MORPHOLOGICAL CHARACTERISTICS

Asporigenous. On solid media, young cells are typically and more or less regularly apiculate; old cells are irregular in shape, from apiculate to ellipsoid, obovate and spheroid, single. Budding very active, normally bipolar. Geant cells scarce or absent, slightly larger than the normal ones. Vegetative cells from 3 to 6 mmm. in diameter or in length, most frequently from 3.5 to 5 mmm. Cells of the ring on liquid media very similar to the preceding, single to aggregated in short chains composed of 2 to 4 cells. Cells of the deposit more frequently round than apiculated, always single, but mechanically aggregated or tangled, mucous; the protoplasm is filled with oil drops and crystalloids; there may be degenerative forms and absence of budding.

BIOCHEMICAL CHARACTERISTICS

It ferments glucose readily but does not ferment levulose, sorbitol, dulcitol, abinose, xylose, rhamnose, mannose, galactose, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin, glycogen and inulin.

It inverts saccharose, but not trehalose; the inversion of raffinose, if any, takes place with difficulty. Strain N. 185 inverts raffinose; other strains do not invert the same hydrocarbonate. Glucose, levulose and saccharose are easily assimilated; galactose and mannose not readily and lactose not to any extent. Assimilates acetic acid well; tartaric, malic and citric acid not readily; methylic and ethylic alcohol not to any extent. Assimilates glycerin very

slowly. Pepton is preferred, but asparagine is assimilated quite well; nitrate of potassium and sulphate of ammonium are assimilated slowly but not nitrite of potassium nor glycocol. Starch is not hydrolized and gelatinase is not produced. This yeast grows apparently without difficulty in media containing 1 per cent of acetic acid.

SYSTEMATIC POSITION

Many tropical species of the genus *Kloeckeria* Janke (showing apiculate, asporogenous cells) were isolated chiefly from the soils and described by Klöcker, but most of them are not completely characterized and easily distinguished, and a comparison with our species cannot be made satisfactorily. The present writer (**5**) described a species of *Kloeckeria* (*K. domingensis* Cif.), isolated from washing water of rotting cacao pods, very rarely found on fermenting cacao beans, and only at the beginning of the fermentation. *K. domingensis* is very distinct from the strains in study, in having larger cells (6–12 by 4–8 mmm.), very regular in shape when young; ferments levulose and glucose only slightly; assimilates glucose, saccharose, levulose and maltose, but not acetic acid; an hydrogen-ion concentration of pH = 5,0–4,6 interrupts the growth. In expectation of the revision of the species of *Kloeckeria* indicated to date, we describe the form in study as a new species: *Kloeckeria cacaoicola* Cif., n. sp.

Strains N. 182, 202 and 254

The strains in study must be referred to the *Kloeckeria domingensis* Cif., previously described (**5**).

Strains N. 158, 163, 167, 184, 197, 199, 210 and 228

Only four strains (N. 158, 163, 184 and 210) were completely studied, the remaining being determined on the basis of cultural characteristics and morphological aspect of the cells on starting medium, as well as on the basis of the fermentation.

CULTURAL CHARACTERISTICS

Optimum temperature for growth: about 35°C.

Starting medium: thin, flat, white to ivory-white, then white-grayish or white-yellowish, mat, more or less regularly rounded colonies, with smooth surface, and smooth plain or irregularly undulate borders.

Gorodkowa's agar: thin, depressed, almost pellicular, poor colonies; cells without ascospores.

Raulin's neutral solution: quick growth, forming a superficial ring at first incomplete, later complete, but irregularly thickened, with a few very small floating islets, but without a well developed velum; easy formation of an

abundant, more or less pulverulent deposit at the bottom of the test tube; the liquid remains clear.

Carrot agar: very abundant development, similar to the growth on the starting medium.

Malt extract agar (geant colony): flat, white to whitish, finally mat, rounded colony, showing a small slightly depressed center (crateriform), surrounded by a peripheric ring; colony thinner at the periphery, with a few not well defined radial striae, but without concentric rings; borders undulate or not, finely ragged in the sub-superficial portion of the colony, but smooth at the surface.

Malt extract water: the same growth as in Raulin's neutral solution, but more abundant at the bottom of the tube; the liquid of culture is slightly decolorized; without any peculiar odor (Plate XXXV, fig. B).

MORPHOLOGICAL CHARACTERISTICS

Young cells cultivated on solid media are generally, ovate or elliptic to elliptic-elongate, with unipolar, rarely bipolar budding, 3 to 5 mmm. in diameter or in length; geant cells not very frequent, more or less rounded, 4 to 6.5 mmm. in diameter not clearly distinct form the normal cells; cells single, rarely aggregated in short chains composed of 2 to 4 cells. Cells of the ring developed on liquid cultures similar to the preceding, but frequently aggregated in chains, straight or branched, composed of 2 to 7 elements; also lengthened, ellipsoid to sub-cylindric cells, 6–9, (generally 6–7 mmm.) by 3–5 mmm., single or chained together with the normal cells. Cells of the small floating fragments of the pellicle completely similar to the preceding, but composed of numerous elongate cells, and a few short normal cells. Deposit composed of more or less rounded to ovate or elliptic cells, with scarce single, buds, showing involutive or degenerative forms, and a more or less disorganized protoplasm. All young cells possess one vacuole, not rarely two or more small vacuoles, and at first one or two fat globules, then many small globules.

BIOCHEMICAL CHARACTERISTICS

Ferments glucose easily and abundantly, not arabinose, xylose, rhamnose, mannose, galactose, levulose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin, and inulin. Fermenting power is very strong in a few strains (as N. 163, 184, 197 and 199), and very reduced in a few other (as N. 184 and 228). Assimilates well glucose, levulose, galactose, maltose and saccharose; also abundantly acetic and citric acid, less tartaric and slightly malic acid; assimilates ethylic alcohol, but not methylic alcohol. It growths very well on peptone solution, and well also in asparagin, nitrate of potassium and sulphate of ammonium solution; the development is poor on nitrite of potassium and on glycocol solutions. Inverts readily saccharose and raffinose, very sparingly trehalose.

SYSTEMATIC POSITION

This form, one of the most frequent asporogenous yeasts, found on fermenting cacao, in spite of the small difference of the cultural and morphologic characteristics, is identical to the "Kahmhefe A"

of Lilienfeld-Toal, found in cacao samples of all countries. In the opinion of the German author, the "Kahmhefe A" is identical, or, at least, very similar to the *"Saccharomyces theobromae"* Preyer, and I agree with him, taking in consideration the necessity of a right interpretation of the so called "spores" of the original description, and the cultural characteristics as referred to the peculiar cultural substratum. For the presence of fat globules in young cells, and the development of the yeast on liquid media according the fundamental form I of Will, this form should be assigned to the genus *Eutorulopsis* Cif. (*Eutorula* Will and not Saccardo). Of the asporigenous yeasts described as species of the genus *Eutorulopsis,* and not producing pigment in culture, two are known: *E. vulgaris* (Will) Cif. & Red and *E. ellipsoidea* (Will) Cif. & Red. The organism studied differs from both strains, in the cultural, morphologic and biochemical standpoint, so that the Preyer's species can be maintained, but transferred to the genus *Eutorulopsis*; *Eutorulopsis theobromae* (Preyer) Cif., n. comb.

Strains N. 157, 171, 187, 198, 222 and 245

Another very common asporogenous yeast, of the same type as the preceding. Only the strains N. 157 and 198 were studied in detail.

CULTURAL CHARACTERISTICS

Optimum temperature for growth, about 40°C.

Starting medium: white-grayish to light-grayish, small, flat, almost pellicular, irregular colonies, very thin, of an uniform thickness or slightly thicker at the borders; borders more or less irregularly sinuate-undulate. Old colonies are gray in color.

Gorodkowa's agar: poor growth, very similar to the preceding, but showing less developed colonies. Not forming spores.

Raulin's neutral solution: quick superficial development, forming a continuous but thin ring and at first cob-webby, then thicker velum; the superficial pellicle is fragmented, but almost entire in old colonies, grayish, finely folded; deposit slimy and abundant, grayish in color; no peculiar odors.

Carrot agar: very similar to the cultures on starting medium.

Malt extract gelatine (geant colony): colony of slow growth, grayish in color; flat, smooth, superficial, forming a great central crateriform depression, without striae, and not well marked concentric rings; borders continous or irregularly and broadly sinuate, in the subsuperficial portion finely striated.

Malt extract water: growth similar to that on Raulin's neutral solution.

One of the most distinctive features is the slow growth of the colonies at the optimum temperatures, in any liquid or solid media (plate XXXV, fig. D and F).

MORPHOLOGICAL CHARACTERISTICS

Cells developed on solid media ovate to elliptic, rarely sub-cylindric or sub-elongate, 2.5–5 by 4–6 mmm., normally 2.5–4 by 3–5 mmm., the longest being 8 mmm. Geant cells not frequent, spheric, 5–7 mmm. in diameter. Cells single or chained (from 2 to 20, rarely more) forming straight or sparingly branched chains; also crown-like aggregation of 10–30 cells are present. Chains and crown are exceptional on solid media cultures, but normal in the superficial velum, and quite frequent in the ring. Budding, unipolar on solid media cultures, and bipolar on the cells of the velum and ring, is very active only on solid media. Cells of the deposit are single or mechanically aggregated by a connective mucosity, or more or less free; they are very scarcely budding, frequently guttulated or vacuolated, showing involutive strange forms. Geant cells are very frequent.

BIOCHEMICAL CHARACTERISTICS

Does not produce gas in arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. It inverts saccharose and raffinose, not at all trehalose, Liquid media containing glucose, maltose, levulose and saccharose are preferred. It develops quite well on acetic acid solution, and in presence of ethylic alcohol, less on tartaric, malic and citric acids, glycerin and methylic alcohol. Peptone solution is preferred, but nitrate of potassium and ammonium sulphate solution are admitted. The liquefaction of the gelatine is very small.

SYSTEMATIC POSITION

This yeast, almost as frequent as the preceding yeast, is easily distinguished from the *Saccharomyces theobromae* by the velocity of the growth, which is rapid in the Preyer's fungus, and slow in this species. In our opinion, this yeast is similar or identical to the "Kahmhefe B" of Lilienfeld-Toal, isolated from Costa Rican and Trinidad cacao beans, but this yeast was not as completely studied by the German author as the other yeasts, so that a complete comparison cannot be made.

This asporogenous yeast must be included in the genus *Eutorula* (*Torula* of the zymologists, p. p.), in spite of presence of chains both straight and branched, crown-like figures, etc. These aggregations, if found both on liquid and solid media, are characteristics of the genus *Blastodendrion* (Ota), which geant colonies are intermediary between the typical geant colonies of the Mycotoruleae and the same of the Torulopsideae. In the fungus at hand, cell aggregations are found almost only on liquid media cultures, and the geant colony is, of course, of the type I of Will (Torulopsideae). At this time the systematic position of the colourless species of the genus *Torulopsis* is so chaotic that an efficient compari-

son is impossible, and therefore we should create a new species "ad interim", which we will name *Torulopsis Lilienfeld-Toalii* Cif., n. sp., dedicated to the distinguished German zymologist Dr. O. A. von Lilienfeld-Toal.

This species differs from the formerly studied yeast by the velocity of the growth, the presence of a complete superficial pellicle, the aggregations of the cellular elements on liquid media cultures, as well as by many biochemical characteristics, chiefly by the absence of the fermentative power.

Strains N. 156 and 234

This yeast is probably of accidental presence in the fermenting cacao.

CULTURAL CHARACTERISTICS

Optimum temperature for growth: about 30°C.

Starting medium: growth rapid; colony at first white, later white-yellowish, dense, creamy, opaque, thick; the center of the colony is smooth and uniform, the border is thinner, grossly and irregularly lobate.

Gorodkowa's agar: very poor growth; single colonies small, round, whitish, flat, partially confluent in a larger colony. No ascospores formation.

Raulin's neutral solution: poorly developed; only fragments of the ring are present, and the deposit, pulverulent and whitish, is not abundant; no pellicle; does not produce peculiar odors.

Carrot agar: characters similar to those observed on the starting medium, but more pronounced; most of the colony run to the bottom of the test tube.

Malt extract gelatin (geant colony): white or whitish, flat, circular, small colony, slowly forming an enlarged central depression, but not of the crateriform type; radial striae or concentric rings are not formed; irregularly sinuate, linear, thin, smooth borders. Geant colony of the Will's fundamental type I.

Malt extract water: as in Raulin neutral solution, but more abundant.

MORPHOLOGICAL CHARACTERISTICS

Does not form ascospores. Cells more or less spheric to spheroid, rarely elliptic to avoid, 3.5–5.5 by 4–6.5 mmm., with unipolar, rarely bipolar budding. Cell single, rarely aggregated in short chains composed of 2 to 4 elements, without complicated aggregations. Geant cells scarce on solid media, more abundant on liquid ones, spheric, 6–8 mmm. in diameter, scarcely distinguished from the normal cells. Cells of the ring spheric to elliptic, 3–5 mmm. in diameter, or 3–4 by 4–6 mmm., normally aggregated in short straight chains composed of 2 to 6 elements; the budding is scarcely active. Cells of the deposit in liquid cultures similar to those on solid media, but with a more or less disorganized protoplasm, frequent involutive forms, and without budding.

BIOCHEMICAL CHARACTERISTICS

Does not produce gas in arabinose, xylose, rhamnose, glucose, mannose, galactose, sorbitol, dulcitol, maltose, lactose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. Inverts saccharose, raffinose, and trehalose. It is not difficult on the selection of sugars as source of carbon: glucose, levulose, maltose and galactose are of the same importance. Does not develop in methylic and ethylic alcohol solutions, as well as on acetic, citric, malic and tartaric acids, and on glycerine. The growth is very abundant on peptone, and scarce or not at all on glycocol, asparagine, potassium nitrate and nitrite and ammonium sulphate. On gelatine cultures, a slow liquefaction takes place.

SYSTEMATIC POSITION

This species is clearly distinct from the preceding, as well as from the *Eutorulopsis* variety isolated from the Dominican cacao. The problem of the comparison of the species in study among the to date more or less completely described species of the genus Torulopsis, remains the same; thus we should create a new species "ad interim", dedicated to Mr. Hamel Smith, pioneer of the study on cacao fermentation, which we will name, *Torulopsis Hamel-Smithii* Cif., n.sp.

Strain N. 161

This strain, isolated together with strains of the *Schizosaccharomyces Bussei,* is indistinguishable by cultural, morphological and biochemical standpoint from this yeast, with the exception of the following characteristics: (1) fails to produce ascospores on Gorodkowa's medium, as well as on chalk cones and on sterilized carrot; (2) the enormous production of arthrospores (or *Oidium*-like cells), chiefly in liquid media; (3) in spite of the absence of the ascospore production, a more or less complete conjugation of the cells may rarely be observed; after the copulation, the cell remains inactive; (4) the fermentative power very reduced, but the same carbohydrates are fermented.

In spite of the fact that the transformation of the *Schizosaccharomyces Bussei* into the asporogenous form or "viceversa" was not experimentally demonstrated, there are no doubts on the relation between ascogenous and anascosporic strains. On the other hand, Lilienfeld-Toal observed the presence of asporogenous strains.

The imperfect form of the genus *Schizosaccharomyces* must be classified under the genus *Schizotorulopsis* Cif., of which only one species is surely known (*S. Alfonsecai* Cif.), not to mention the *S. asporus* Eykman and named: *Schizotorulopsis Bussei* Cif., n. nom.

Strain N. 215–215 bis.

This strain was isolated for the first time in January 1927 from fermenting cacao from a cacao estate located at La Vega. It was generically determined, and the failure to produce fermentation ascertained. Before the transplant of the month of July, the strain died. A new casual reisolation was made during the month of October of the same year from a fermenting cacao of a cacao farm of Moca, and the study was completed without delay. Five months after the reisolation, I also lost the second strain.

CULTURAL CHARACTERISTICS

Optimum temperature for growth: about 40–42°C.

Starting medium: white or whitish, creamy, slimy, not abundant colonies, of slow growth also at the optimum temperature, and very slow at lower temperatures; the colonies are smooth, glassy, without distinctive particularities.

Gorodkowa's agar: very small, lenticular, alabastrine, scattered colonies. Not forming spores.

Raulin's neutral solution: very reduced development, both superficial and at bottom; only small and scattered fragments of rings are formed; no superficial pellicle; very scarce, slimy, white-yellowish deposit.

Carrot agar: of the cultural subtrata tested, this agar appears as the best; colony very similar to those on starting medium, but more abundant.

Malt extract gelatine (geant colony): very small, round, flat, white, smooth colony, without crater, rings or striae; borders plain, smooth, uniform. The type of the colony is the I type of Will.

Malt extract water: development similar to that on Raulin's neutral solution, but slightly abundant; the ring is more developed.

MORPHOLOGICAL CHARACTERISTICS

Cells developed in cultures on solid media clearly biform; most frequently sphaeric to subovate, about 4 to 6 mmm. in diameter, mixed with elliptic-lengthened or cylindroid cells, of 7–12 by 5–8 mmm. The multiplication effected is by a process beginning by budding and ending by fission, as described in the genus *Schizoblastosporion*. Cells of the ring on liquid media cultures similar to the preceding but most abundant cells are the elongate instead of the rounded. Cells of the deposit generally spheric, frequently showing degenerative forms, with a more or less spoiled protoplasm; geant cells present but scarce, not easily distinct from normal spheric cells but for a thicker membrane and a slightly greater (diameter 5–7.5 mmm.).

BIOCHEMICAL CHARACTERISTICS

They do not ferment arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. Does not invert saccharose, trehalose and raffinose. Assimilates abundantly only glucose and levulose, not maltose, saccharose, mannose, galactose and lactose, nor organic acids, or alcohols. Growth good only on peptone solution. Fails to liquefy gelatine; does not

produce sulphuretted hydrogen from powdery sulphur; fails to produce indol or trytophane, and does not give the biuret reaction; does not hydrolize starch; methylene blue solution is decolorized very slowly.

SYSTEMATIC POSITION

This strain falls under the genus *Schizoblastosporion,* a genus of asporigenous yeasts characterized by the reproduction by budding-fission. The only species to date described is the type of the genus: *S. Starkeyi-Henricii* Cif. (**5**), isolated from North American soil. Under the denomination "Hefe R", Lilienfeld-Toal isolated from cacao of St. Thomé another strain of Torulopsidacea, undoubtedly pertaining to the same genus *Schizoblastosporion.* Differential characteristics between *S. Starkeyi-Henricii,* "Hefe R", and our strain are summarized in the following table.

Schizoblastosporion Starkeyi-Henricii	*"Hefe R" of Lilienfeld-Toal.*	*Strains N. 215-215 bis of Ciferri.*
Apparently of strong vitality.	In culture 50% of the cells dead.	of feeble-vitalty.
White colony.	Yellowish colony.	White or whitish colony.
No pellicle.	Mucous pellicle.	No pellicle.
Rapid growth.	Slow growth.	Slow growth.
?	Liquefy gelatine.	Not liquefy gelatine.
?	Optimum temp. 20°C.	Optimum temp. 40-42°C.
Cells not very mucous.	Cells very mucous.	Cells not very mucous.
Spheric cells 2-7 mmm. of diameter.	Spheric cells 6, 5-7 mmm. of diameter.	Spheric cells 4-6 mmm. of diameter.
Elongate cells 10-22 by 5.5-7 mmm.	Elongate cells 10 by 20 mmm.	Elongate cells 7-12 by 5-8 mmm.
Without geant cells.	With geant cells.	With geant cells.
Glucose is preferred.	Glucose is preferred.	Glucose and levulose are preferred.

These differences are, in our opinion, distinguishing the "Hefe R" from the strains N. 215–215 bis, and both from *S. Starkeyi-Henricii,* basing our opinion on the cultural, morphological and biochemical differences above summarized. According to these conclusions, we propose the binomial *Schizoblastosporion santhomensis* Cif., n. nom. for the Lilienfeld-Toal's "Hefe H", and *Schizoblastosporion domingensis* Cif., n. sp. for the strains N. 215–215 bis.

In closing, from the study direct or indirect of two other species of the same genus, we are able to perfect the generic description of *Schizoblastosporion* Cif.: asporogenous yeast, in which multiplication begins by budding and ends by fission; frequently of feeble vitality; without fermentative power; assimilation of carbon and azote generally specialized from one or a few determined compounds; cells always bimorphe, the smaller spheric and the longer elliptic to cylindric.

STRAIN N. 216

Isolated only once from dry fermented cacao beans, but here

very abundant. This strain must be considered as the *Torula aurantiaca* Saito (**25**), isolated from the air in Manchury. This species being well known from Saito's description, we are adding only the characteristics not previously observed, or different from those of the Japanese author.

CULTURAL CHARACTERISTICS

Agar cultures are generally abundant, easily developed, smooth, with plain or irregular, simple borders. Gelatine cultures of the same type of all simple Torulopsidaceae (I fundamental type of Will), with a small central depression, and simple, and smooth borders. Cultures in liquid media forming a small, rather incomplete ring, and a more or less abundant deposit, but not pelliele. Optimum of temperature for growth about 30°C. Cells normally not aggregated in chains, and not forming arbuscula, crowns, and so on. Does not ferment arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose trehalose, raffinose, starch, soluble dextrin and inulin. Does not invert raffinose. Develops abundantly on glucose, levulose, maltose and galactose, as well as peptone, less on asparagine, nitrate of potassium and ammonium sulphate.

SYSTEMATIC POSITION

This species is reported for the first time found outside of the type locality, and probably, accidentally present on fermenting cacao, should be transferred to the genus Torulopsis as *Torulopsis aurantiaca* (Saito) Cif. & Red.

Strain N. 204

This strain was isolated only one time, and as the preceding strain, must be considered as an accidental contamination of the fermenting cacao beans. It may be considered as identical with the *Torulopsis mucilaginosa* (Jörgens.) Cif. & Red., a pinkish Torulopsidea, described by Jörgensen (**16**) and revised and reclassified by the present writer and Redaelli (**8**).

Strain N. 153

As the preceding two strains, but exceedingly frequent on the sample examined. In our opinion, this strain is a variety of the *Torula rumosa* Saito (**25**), a Mycotorulea, that, according the description of the Japanese author, as well as the observation on the strain at hand, must be referred to the genus *Mycotorula* Will as *Mycotorula ramosa* (Saito) Cif., n. comb. Only differential characteristics from Saito's description are indicated.

CULTURAL CHARACTERISTICS

On favorable solid media the colonies are abundant, at first smooth, then with plume-like borders, and with an abundant sub-superficial development of root-like mycelium. The borders are finely engraved to dentate, and under the microscope, a dense, net-like work is visible. On gelatine, the geant colony is clearly sulcate by radial, simple or branched, striae, starting from a depressed to crateriform center to the periphery. No or only sketchy concentric rings are visible. Borders dentate and plume-like, with a dense sub-superficial mycelium vegetation. On liquid media, the development is abundant, forming at first a complete and generally dense ring, then a serie of floating islets, finally aggregated into an orange-reddish, finely folded, mat superficial pellicle. The deposit is ropy, brownish in color. The color of the colonies on solid media oscillate form gold-yellow to orange-reddish.

MORPHOLOGICAL CHARACTERISTICS

The morphology of the cell is very similar to that described by Saito, but the cells of the velum reach 30 mmm. in length and 6.5 mmm. of thickness. Geant spheric cells are frequent in the deposit of liquid cultures, being from 6 to 9 mmm. in diameter.

BIOCHEMICAL CHARACTERISTICS

Does not ferment arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. Inverts trehalose, saccharose and raffinose. Liquefies gelatine slowly. Growth good on glucose, saccharose, multose, but not on levulose and galactose. Only the peptone is favorable. The optimum of the temperature for growth is lower than 30°C.

SYSTEMATIC POSITION

This strain differs from the *Mycotorula ramosa* in many particulars of the colonies both on solid and liquid media, as well as in the morphology of the cells and in the biochemical characteristics. For this variety we propose the name: *Mycotorula ramosa* (Saito) Cif. var. *dominicana* Cif., n.var.

STRAINS N. 160, 162, 164, 170, 176, 177, 188, 192, 214 and 227

All these strains are referable to the genus *Geotrichum* (*Mycoderma* of Auct. sensu stricto, or *Oidium* of the zymologists, pro parte). With the exception of the strains N. 164, 177 and 192, all others are not easily distinguished from the morphological and biochemical points of view, while cultural characteristics are but little differentiated. For brevity, the most outstanding characteristics only are summarized.

Strain N. 160

CULTURAL CHARACTERISTICS

On solid favorable media, it forms a poorly developed, folded, white, pellicular colony in which borders are more or less plume-like. In favorable liquid media, the ring is fused to a well developed, white-folded, thin, dusty, at last floating pellicle; the deposit is abundant and slimy. The geant colony on malt extract agar is thin, pellicular, finely but indistinctly folded, chiefly at the borders, but without concentric rings. This yeast does not liquefy the gelatine.

Strain N. 162

CULTURAL CHARACTERISTICS

On solid favorable media, it forms an abundant, white-grayish to white-yellowish mat, thick, at first smooth, then more or less woolly, irregularly but very densely cerebroid or mesentheroid-folded colonies. The borders are thick, almost smooth. In favorable liquid media, the pellicle is abundant, yellowish, cerebriform-sulcate, dense; the ring is fused with the pellicle. The geant colony on malt extract agar is thick, irregularly cerebriform-convolute, with a broad central depression. The gelatine is not liquefied, or very slow.

Strain N. 164

All cultural characteristics are similar to those of the strain N. 160, but the colonies on solid media are more developed.

Strain N. 170

Very similar to the strain N. 160; the deposit is mucous more than slimy, and the geant colony shows more well marked radial striae and almost indistinct concentric rings.

Strain N. 176

Similar to the strain N. 162, but thinner and yellowish in color.

Strain N. 177

CULTURAL CHARACTERISTICS

Very abundant, whitish, dry, plain, abundant and of rapid growth colonies, covered by a dense cottony layer, when developed on solid favorable media. In liquid media, the velum is cottony or densely wool-like, but irregularly developed, almost white in color, at last floating. The geant colony on malt extract agar is very well developed, almost hirsute, under the superficial cotton-like layer, crenulate, dry; the borders are uniform and plain. The center is somewhat prominent, but irregularly, and one or few not well marked concentric rings are visible. The gelatine is not liquefied.

Strain N. 188

Similar to the preceding, but with well marked radial striae, and more or less indistinct concentric rings. The colonies on solid media are more developed, and a slow but diffused liquefaction of the gelatine takes place. The colonies are always smooth, never crenulate.

Strain N. 192

Not distinct from the strain N. 188.

Strain N. 227

Very similar to the strain N. 162.

Strains N. 160, 162, 164, 170, 176 177, 188, 192 214 and 227.

MORPHOLOGICAL CHARACTERISTICS

Very similar in all studied strains. Arthrospore generally from 3.5 to 6 mmm. by 5 to 12 mmm.; mycelic hyphae more or less slender, branched, septate, of the same thickness. Strains N. 160, 164 and 170 have only creeping hyphae; strains N. 162, 176 and 227 abundant creeping hyphae, but also a few, short and poorly developed, sub-erect, hyaline, sparingly branched hyphae; strains N. 177, 188 and 192 possess creeping hyphae as well as erect, densely branched, cottony, septate, apparently true myceliar hyphae. All creeping hyphae easily produces chained arthospores; sub-erected hyphae may produce arthrospores only in contact with the solid substrata; erected, true myceliar hyphae; apparently never produces arthrospores.

BIOCHEMICAL CHARACTERISTICS

The most outstanding difference is the power of liquefying the gelatine. Studied strains do not ferment any tested carbohydrate; growth good on glucose, saccharose, maltose, and levulose; less on galactose; ethylic alcohol, as well as acetic and citric acid (less on tartaric and malic) are accepted; not so well glycerine; not to any extent on methylic alcohol. Peptone, asparagine, nitrate of potassium and sulphate of ammonium are easily accepted; not so well glycocol; not or very little on nitrite of potassium.

SYSTEMATIC POSITION

Up to the present time the species of the genus *Geotrichum* are generally grouped in a few, more or less distinct species, or included under comprehensive determination that is so bewildering that a comparison with our strains cannot be made. For this reason we prefer the temporary grouping into species and variety on the base of the tested differences, according to the following key:

A. Cerebriform-sulcate colonies, more or less woolly, whitish to yellowish, slowly or not liquefying the gelatine (Strains N. 162–type, N. 176 and 227.)—*Geotrichum cerebrinum* Cif. n. sp.

B. Pellicular, smooth, poorly developed colonies, whitish to yellowish, not liquefying the gelatine (Strain N. 160–type, N. 164 and N. 170).—*Geotrichum flexuosum* Cif., n. sp.

C. Colonies showing striae and concentric rings, covered by a cottony layer, white to grayish in color.

a. Crenulate colony; not liquefying gelatine (strain N. 177).—*Geotrichum byssinum* Cif., n. sp.

b. Smooth colonies, liquefying the gelatine (strain N. 188–type and N. 192).—*Geotrichum byssinum* Cif. var. *rigidum* Cif. n. var.

YEASTS FOUND IN SANTO DOMINGO AS COMPARED TO THE YEASTS FOUND IN OTHER COUNTRIES

The yeasts common to Santo Domingo and other tropical countries, as found on fermenting, or fermented cacao beans, are the following:

1. *Saccharomyces ellipsoideus* var. *tropicus* Lil.–Toal & Henneb.—Intertropical.
2. *Endomyces anomalus* (Hans.) Zender.—Intertropical.
3. *Schizosaccharomyces Bussei* Lil.–Toal & Henneb.—Intertropical.
4. *Eutorulopsis theobromae* (Preyer) Cif. ("Kahmhefe A" Lil.–Toal).—Intertropical.
5. *Schizotorulopsis Bussei* Cif.—Intertropical.

The following yeasts are common to Santo Domingo and other countries, but not tropical countries, nor on fermenting or fermented cacao beans:

6. *Torulopsis aurantiaca* (Saito) Cif. & Red.—Manchury.
7. *Torulopsis mucilaginosa* (Jörg.) Cif. & Red.—Europe.

The following yeasts are found only in Santo Domingo, on the same substratum:

8. *Saccharomyces ellipsoideus* var. *domingensis* Cif.
9. *Kloeckeria cacaoicola* Cif.
10. *Kloeckeria domingensis* Cif.
11. *Torulopsis Lilienfeld-Toalii* Cif.
12. *Torulopsis Hamel-Smithii* Cif.
13. *Schizoblastosporion domingensis* Cif.
14. *Mycotorula ramosa* (Saito) Cif. var. *dominicana* Cif.
15. *Geotrichum cerebrinum* Cif.
16. *Geotrichum flexuosum* Cif.
17. *Geotrichum byssinum* Cif.
18. *Geotrichum byssinum* Cif. var. *rigidum* Cif.

The following yeasts, not found in Santo Domingo, are described on fermented cacao of other tropical countries:

19. *Saccharomyces ellipsoideus* Hans. var. *brasiliensis* Cif. (= "Weinhefe B" Lil.–Toal).—Brazil
20. *Schizoblastosporion santhomensis* Cif. (="Hefe R" Lil.–Toal).—San Thomé.
21. *Torulopsis neotropica* (*) Cif., n.nom. (="Kahmhefe B" Lil.–Toal).—Costa Rica and Trinidad.
22. *Saccharomyces theobromae-fermentans* Cif., n.nom. (**) (="Saccharomyces M" Lil.–Toal).—Trinidad.

THE FERMENTATION OF THE CACAO AND THE YEAST DISTRIBUTION

The distribution of the yeasts in relation to the course of fermentation may be deduced from the isolations made during the experiments. Judging from the strains isolated, we may divide the yeasts in: (1) yeasts normally present during the fermentation of cacao, and (2) yeasts of accidental or occasional presence. The yeasts of the first category, and, of course, that may play a role in the fermentation, are: (1) *Saccharomyces ellipsoideus* var. *tropicus*, (2) *S. ellipsoideus* var. *domingensis*, (3) *Endomyces anomalus*, (4) *Schizosaccharomyces Bussei*, (5) *Kloeckeria cacaoidola*, (6) *Eutorulopsis theobromae*. Less frequent, but not of exceptional presence, are: (1) *Torulopsis Lilienfeld–Toalii* and (2) *Kloeckeria domingensis*. Accidentally found on fermenting cacao beans are: (1) *Torulopsis Hamel-Smithii*, (2) *Schizoblastosporion domingensis*, (3) *Torulopsis auranticaca*, (4) *Torulopsis mucilaginosa*, (5) *Mycotorula ramosa* var. *dominicana*, (6) *Geotrichum cerebrinum*, (7) *G. flexuosum*, (8) *G. byssinum*, (9) *G. byssinum* var. *rigidum*.

In relation to the frequency of each species or variety of yeast, and the period of fermentation, including the period of post-fermentation, we may consider four phases, namely: (1) beginning of fermentation, (2) fulness of fermentation, (3) end of fermentation, (4) persistance on dry fermented cacao beans, conserved at the normal conditions, six months after the fermentation. In the scheme of the yeasts distribution, the absence of the species is expressed

(*) From the description of Lilienfeld-Toal, it is almost impossible to arrange this yeast in one of the genera of the asporigenous. As species of *Torulopsis* it is not typic of the genus.

(**) According to the German author above quoted, this yeast is similar both to *Willia anomala* and *Saccharomyces lactis* Dombrowski, but it is apparently more like the *Saccharomyces fragilis* Jörg. It differs from the three above quoted species by many cultural, biochemical and morphological characters, and may be a distinct and new species.

by 0; + indicated very rare; ++ quite rare; +++ common; ++++ very common.

Yeast	Beginning of fermentation	Fulness of frementation	End of fermentation	Dry beans
Saccharomyces ellipsoideus var. tropicus	++	++++	+++	+++
S. ellipsoideus var. domingensis	++	+++	+++	+++
Endomyces anomalus	+++	++	++	++
Schizosaccharomyces Bussei	+	+++	+++	++
Kloeckeria cacaoicola	+++	++	+	0
Eutorulopsis theobromae	+++	++++	++++	+++
Torulopsis Lilienfeld-Toalii	++	+++	++++	+
Kloeckeria domingensis	+++	+	0	0
Torulopsis Hamel-Smithii	++	0	0	0
Schizoblastosporion domingensis	+	0	0	0
Torulopsis aurantiaca	0	0	+	0
Torulopsis mucilaginosa	0	0	0	+
Mycotorula ramosa var. dominicana	0	0	+	0
Geotrichum cerebrinum	0	0	+	+
G. flexuosum	0	0	+	+
G. byssinum	0	0	0	+
G. byssinum var. rigidum	0	0	+	0

KEY TO THE GENERA, SPECIES AND VARIETIES OF CACAO YEASTS

A. Sporogenous yeasts *(Saccharomycales)*.

I. Without true mycelium (*Saccharomycetaceae*).

1. Multiplication by budding (*Saccharomyces* Meyen).

a. The ellipsoid type (*Saccharomyces ellipsoideus* Hansen).

b. Fermenting glucose, raffinose, levulose, saccharose, maltose and galactose. — *S. ellipsoideus* Hans. var. *tropicus* Lil.-Toal.

bb. Fermenting glucose, raffinose, maltose, saccharose, levulose but no galactose.—*S. ellipsoideus* Hans. var. *domingensis* Cif.

bbb. Fermenting glucose and galactose only.—*S. ellipsoideus* Hans. var. *brasiliensis* Cif.

c. Not so.—*S. theobromae-fermentans* Cif.

2. Multiplication by fission (*Schizosaccharomyces* Linder).

Only species.—*S. Bussei* Lil.-Toal & Henn.

II. With a true mycelium (*Endomycetaceae;* genus *Endomyces* Rees).

Only species.—*E. anomalus* (Hans) Zender.

B. Asporogenous yeasts (*Atelosaccharomycetaceae-Torulopsilaceae*).

I. Without a true mycelium (*Torulopsideae*).

1. Multiplication by budding.

a. Cells spheric, ovate to ellipsoid.

b. Young cellules with one or more fatty corpuscles; growing according to Will's fundamental form I (*Eutorulopsis* Cif.)

Only species.—*E. theobromae* (Preyer) Cif.

bb. Young cellules without fatty corpuscules, growing according to Will's fundamental form III (*Torulopsis* Berl. emend).

c. Colonies pink or red to orange in color.

d. Colonies orange in color, developing abundantly in glucose, levulose, maltose and galactose solution.—*T. aurantiaca* (Saito) Cif. & Red.

dd. Colonies pinkish, developing abundantly in saccharose solution only.—*T. mucilaginosa* (Joerg.) Cif. & Red.

cc. Colonies whitish, yellowish or grayish in color.

d. Cellular elements aggregated in liquid media, producing a complete superficial pellicle.—*T. Lilienfeld-Toalii* Cif.

dd. Cellular elements not aggregated or only shortly chained, not producing a complete pellicle or without pellicle.

e. Cells chiefly ovate, colonies grayish, with lacerated borders.—*T. neotropica* Cif.

ee. Cells chiefly spheric, colonies white-yellowish, with linear borders.—*T. Hamel-Smithii* Cif.

aa. Cells generally apiculated or lemon-shaped.—(*Kloeckeria* Jancke.)

b. Cells large, fermenting levulose and glucose, not growing in pronounced acid media.—*K. domingensis* Cif.

bb. Cells smaller, fermenting glucose, growing in pronounced acid media.—*K. cacaoicola* Cif.

2. Multiplication by fission (*Schizotorulopsis* Cif.)
Only species.—*S. Bussei* Cif.

3. Multiplication starting by budding and ending by fission.—*Schizoblastosporion* Cif.)

a. White or whitish colonies, not liquefying gelatine, optimum temperature about 40–42°C. Cells small.—*S. domingensis* Cif.

aa. Yellowish colonies; liquefying, optimum temperature about 20°C. Cells larger.—*S. santhomensis* Cif.

II. With a true mycelium (*Mycotoruleae*).

1, With or without occasional arthrospores (*Mycotorula* Will.) One species.—*M. ramosa* (Saito) Cif.

2. Elongated or rectangular long chained arthrospores (*Geotrichum* Link).

a. Cerebriform-sulcate colonies, more or less woolly.—*C. cerebrinum* Cif.

aa. Pellicular, smooth colonies.—*G. flexuosum* Cif.

aaa. Cottony colonies, showing concentric rings and striae.

b. Colonies crenulated, not liquefying gelatine.—*G. byssinum* Cif.

bb. Colonies smooth, liquefying gelatine.—*G. byssinum* Cif. var. *rigidum* Cif.

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Explanation of Plates

Plate XXVIII. Loss in weight of fermented cacao and unfermented cacao exposed to the sunshine.

Plate XXIX. Increase in weight of fermented and unfermented cacao beans exposed to saturated water vapor.

Plate XXX. Increase in weight of fermented and unfermented cacao beans dried at differented temperatures and exposed to saturated water vapor.

Plate XXXI. Daily variations of weight of fermented and unfermented cacao beans in relation to the average of the atmospheric humidity.

Plate XXXII. Hourly variation of weight (increase and loss) of fermented and unfermented cacao beans exposed to the free air.

Plate XXXIII. Sketch of the Wilson's distillation box for the desiccation of the cacao beans.

Plate XXXIV. Five days record of a double recording thermograph, showing the temperature of the Wilson box as compared to the air temperature.

Plate XXXV.

Figure A. *Geotrichum byssinum* Cif. var, *rigidum* Cif. Old geant colony on malt extract gelatine (natural size).

Figure B. *Eutorulopsis theobromae* (Preyer) Cif. Adult geant colony on malt extract agar (natural size).

Figure C. *Schizosaccharomyces Bussei* Lil.—Toal & Henn. Old superficial vegetation on malt extract agar (one-half natural size).

Figure D. *Torulopsis Lilienfeld-Toalii* Cif. Adult geant colony on malt extract agar (natural size).

Figure E. *Geotrichum byssinum* Cif. Young but well developed superficial vegetation on malt extract agar (more than half natural size).

Figure F. *Torulopsis Lilienfeld-Toalii* Cif. Photograph of very young cellular aggregation on starting medium (very much magnified).

Figure G. *Geotrichum cerebrnium* Cif. Young colony on starting medium (about ¾ natural size).

Figure H. *Geotrichum byssinum* Cif. Young geant colony on malt extract agar (natural size).

Figure I. *Geotrichum byssinum* Cif. Old geant colony on malt extract agar (natural size).

Figure L. *Geotrichum byssinum* Cif. Young colony on carrot agar (natural size).

Figure M. *Geotrichum flexuosum* Cif. Very old geant colony on malt extract agar (natural size).

PLATE XXVIII.

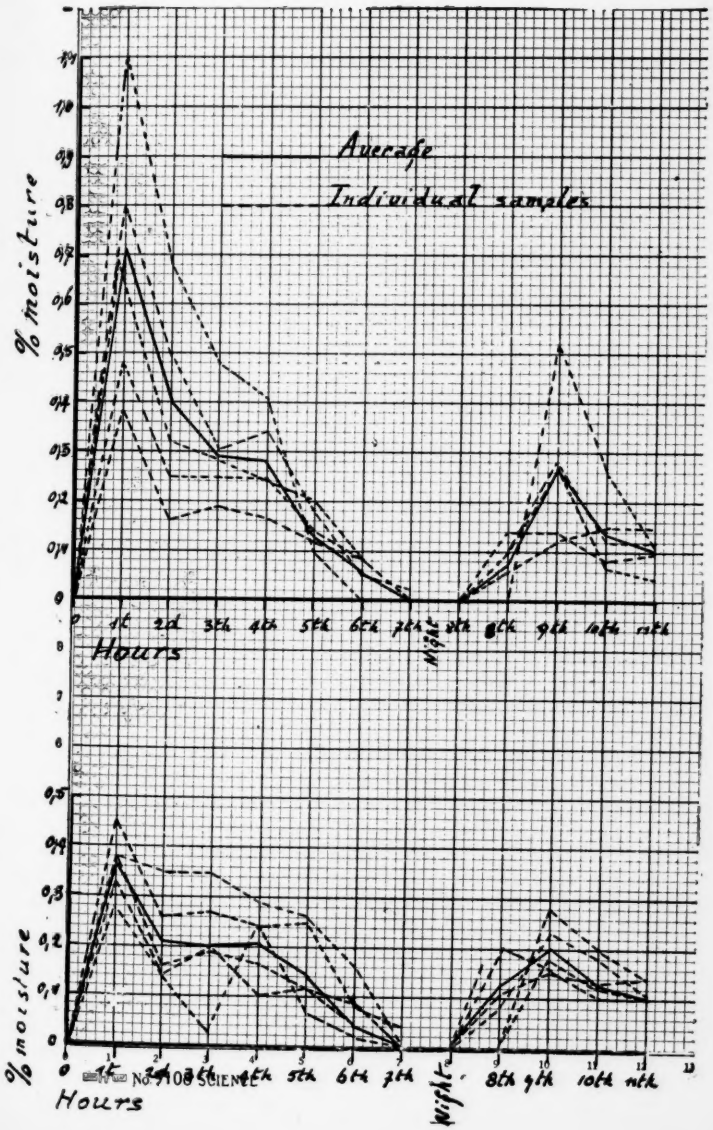


PLATE XXIX.

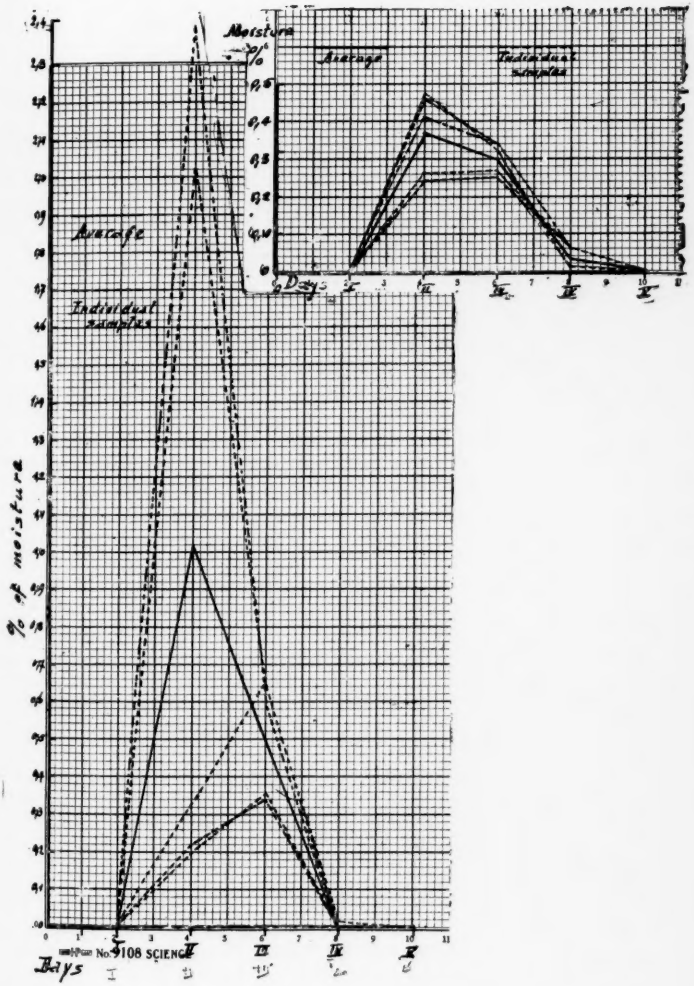
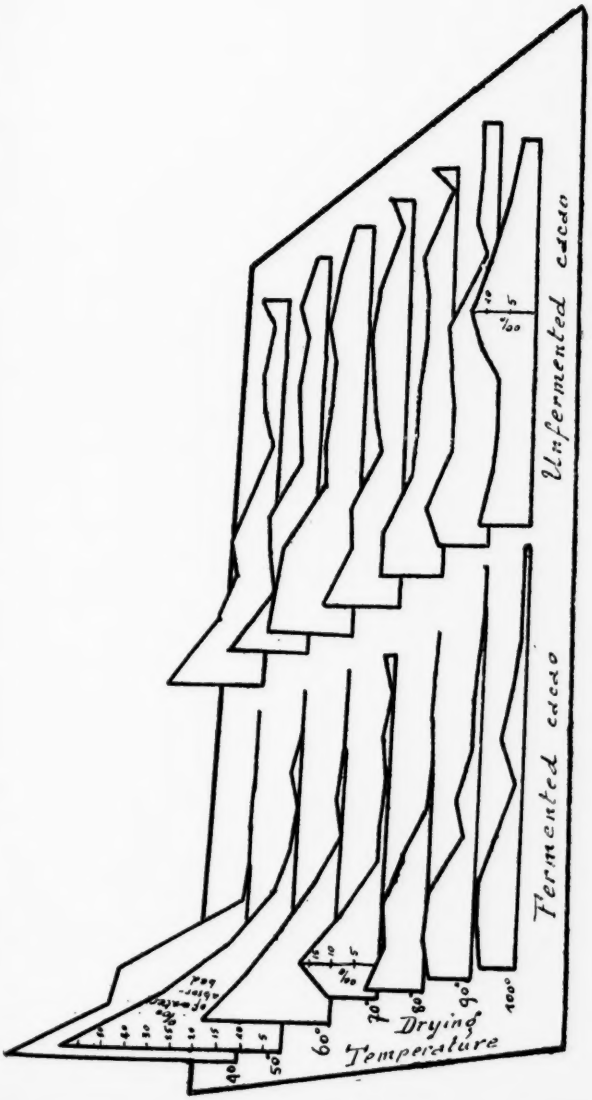
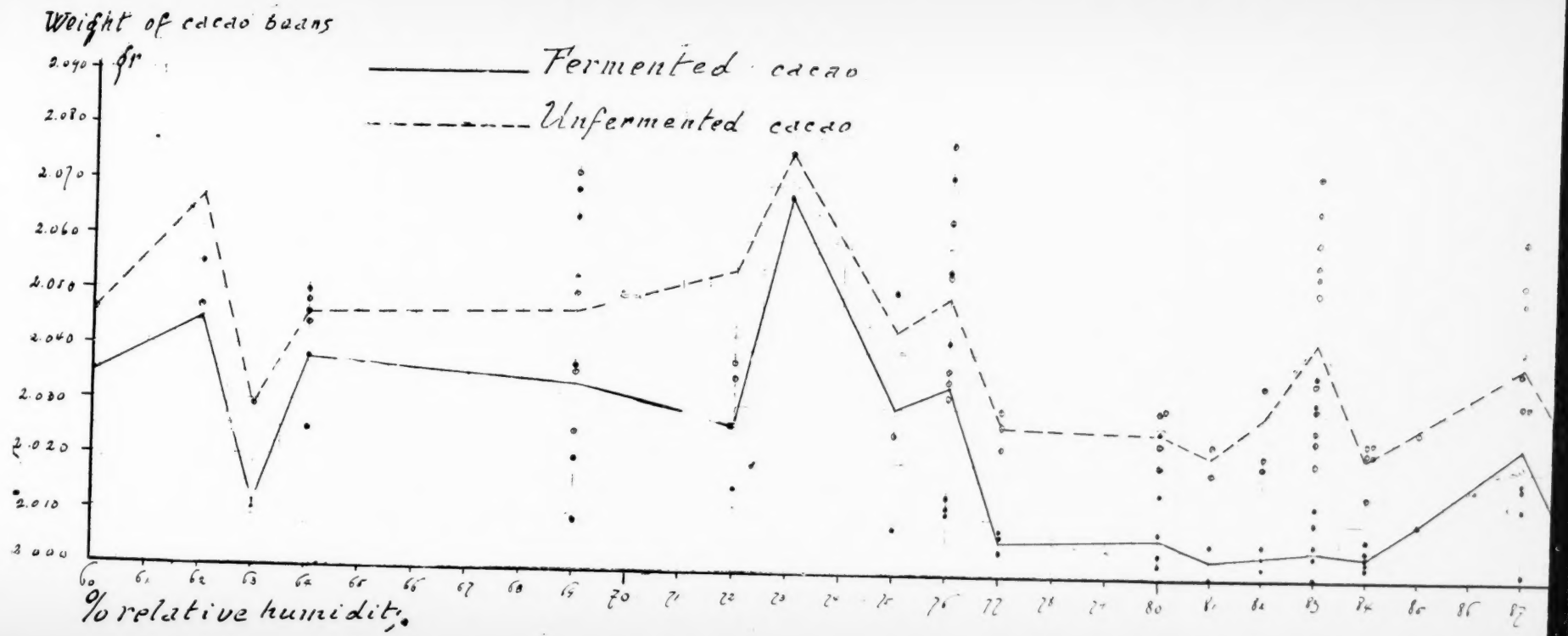

Moisture
%
Average
Individual samples
Days
% of moisture
Individual samples
Average
Form No. 9108 SCIENCE
Days

PLATE XXX.


Unfermented cacao
Fermented cacao
Drying Temperature
40°
50°
60°
70°
80°
90°
100°


Weight of cacao beans
gr
Fermented cacao
Unfermented cacao
2.090
2.080
2.070
2.060
2.050
2.040
2.030
2.020
2.010
2.000
60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87
% relative humidity.

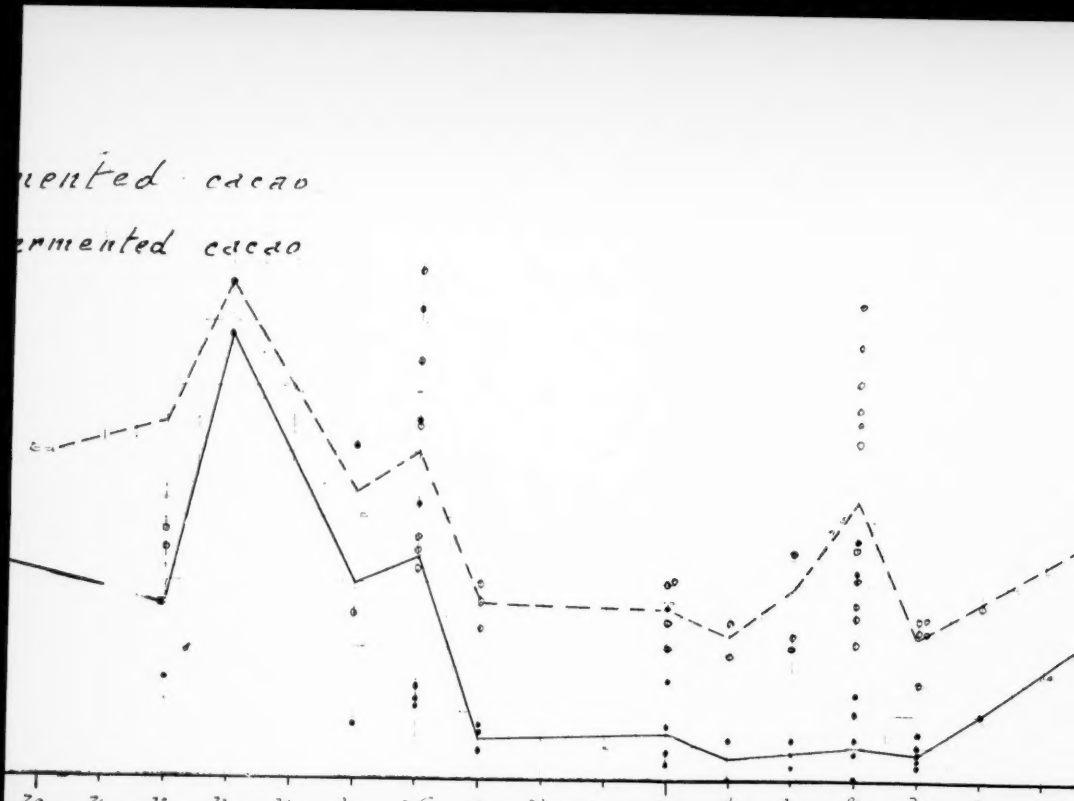

ented cacao
rmented cacao

PLATE XXXI.

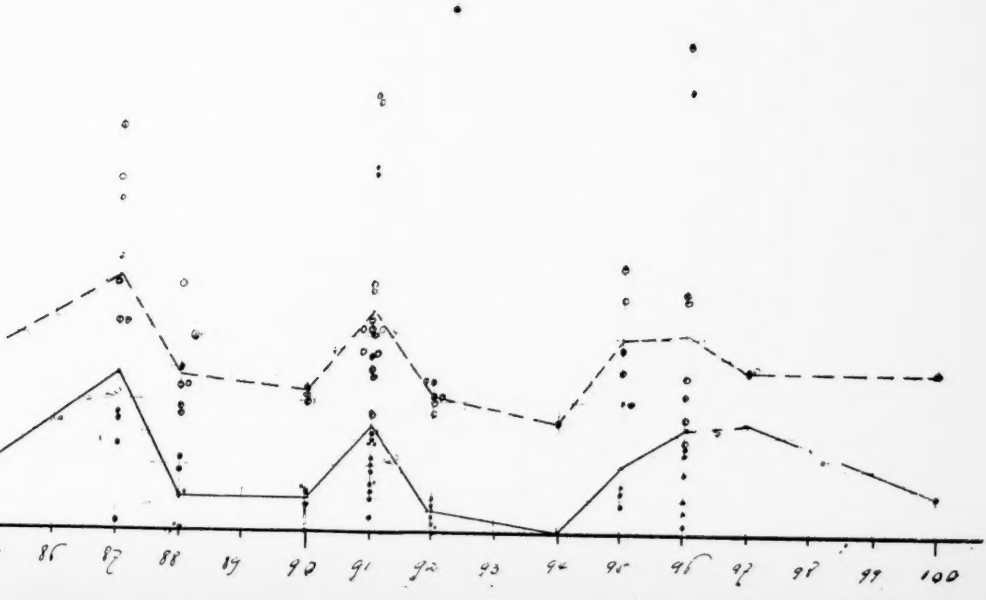
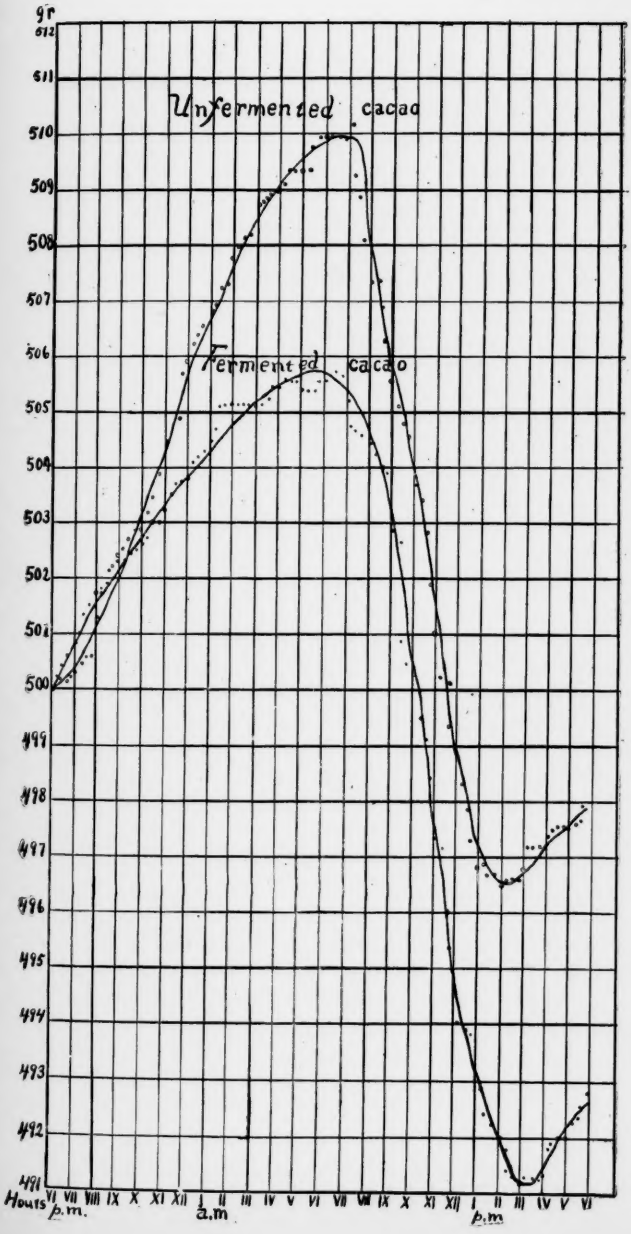


PLATE XXXII.


gr
512
511
510
509
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507
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504
503
502
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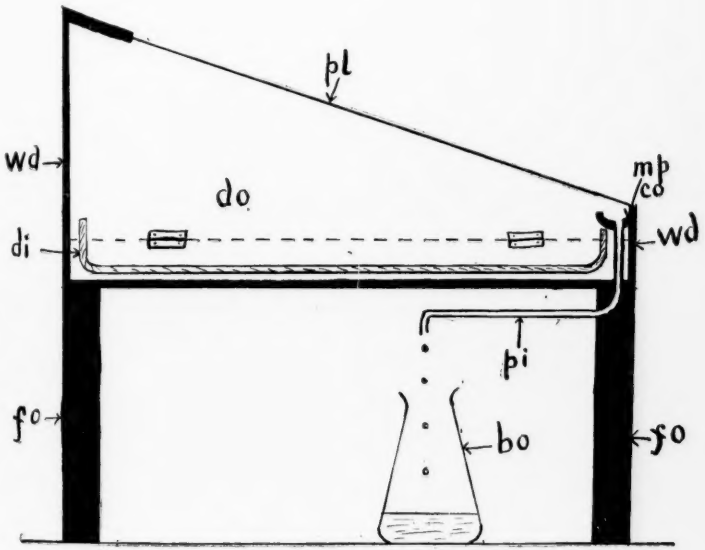
497
496
495
494
493
492
491
Unfermented cacao
Fermented cacao
Hours
VI VII VIII IX X XI XII I II III IV V VI VII VIII IX X XI XII I II III IV V VI
p.m.
a.m
p.m

PLATE XXXIII.



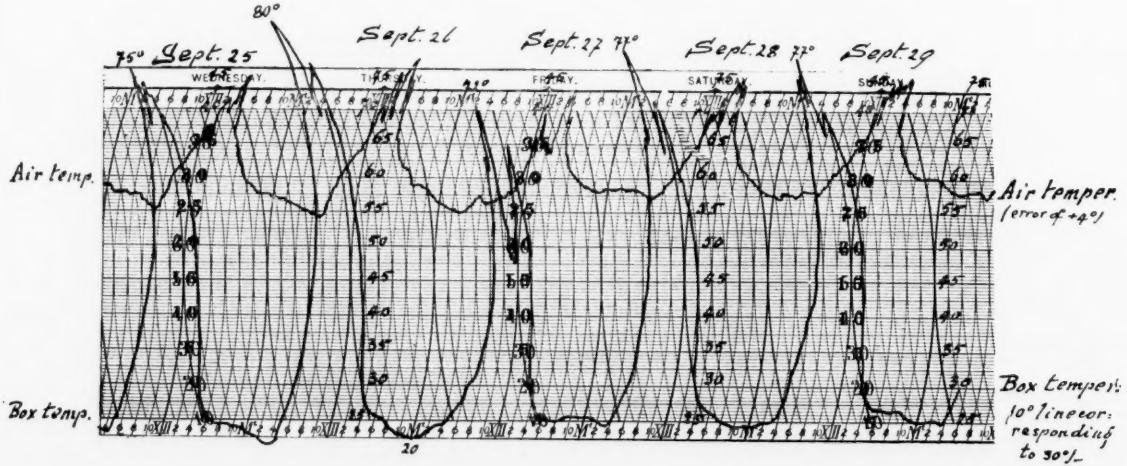
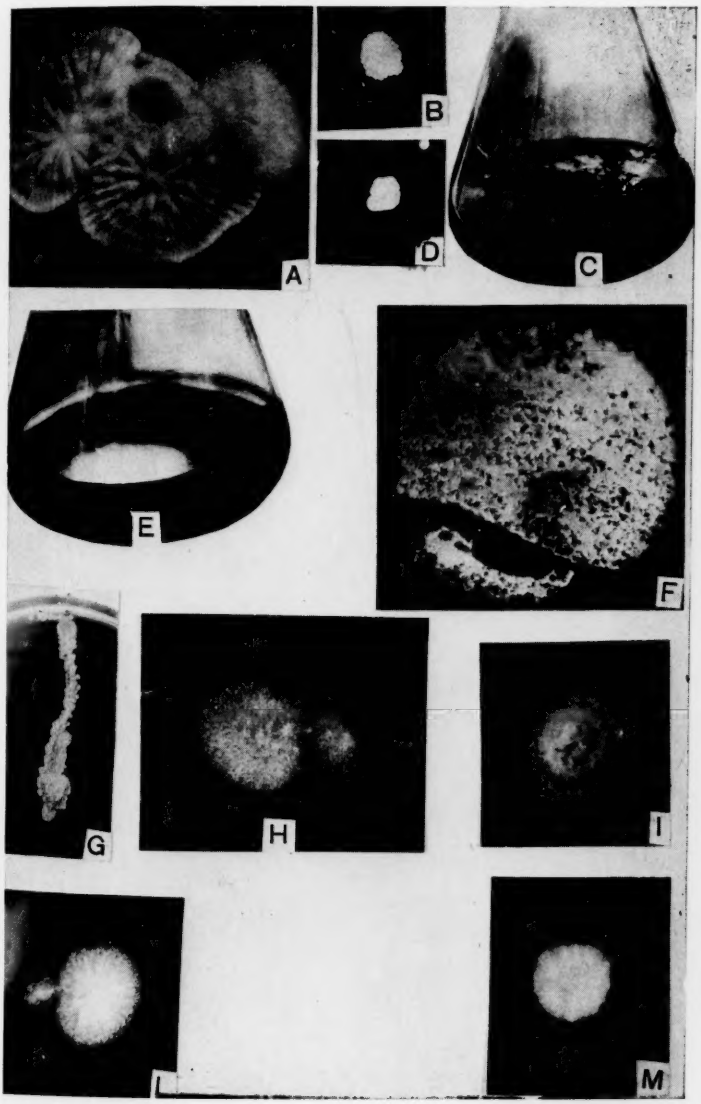

80°
75° Sept. 25
Sept. 26
Sept. 27 77°
Sept. 28 77°
Sept. 29
WEDNESDAY.
THURSDAY.
FRIDAY.
SATURDAY.
SUNDAY.
Air temp.
Box temp.
Air temper.
(error of +4°)
Box temper:
10° lineor:
responding
to 30°/_
20

PLATE XXXIV.

PLATE XXXV.



STUDIES ON THE BACTERIAL WILT OF THE SOLANACEAE IN PORTO RICO

J. A. B. NOLLA *

A bacterial disease of solanaceous crops has been prevalent in Porto Rico for many years, the first apparent report on the occurrence of this disease, having been made by Henricksen in 1906 (**3**). Outbreaks of this malady in solanaceous vegetable crops have been very severe. Since the demand for these crops had been limited to local consumption the importance of the disease was not fully appreciated until 1926 when shipments of tomatoes, peppers, eggplants and other vegetables began to be made to the United States. No sooner was planting of these solanaceous plants extended than the losses from this disease increased very materially. It was then recognized that, if the growing of tomatoes, eggplant and peppers was to be safeguarded against serious diseases, a study of the bacterial wilt was necessary. The writer was asked in the autumn of 1926 to undertake an investigation of the malady with special reference to its control. It is obvious that certain other points had to be studied to some extent in order to gain a better knowledge of the relations between the pathogen and its suscepts such as would clear the way for the all-important subject of control.

SUSCEPTS

VARIETAL SUSCEPTIBILITY

As a knowledge of the susceptibility of solanaceous crops is indispensable, preliminary trials were made in the growing season of 1926–27 with commercial varieties of tobacco, peppers, tomatoes and eggplant. The potato was added later, as information on the susceptibility of this suscept seemed important, since certain trials had shown it to be a promising new crop for the Island. Seed of all these crops was obtained from various sources in the United States.

The susceptibility of varieties of pepper, eggplant and tomato was determined by seed-bed tests and by studies in the field after transplanting. It was observed during the first year's work that the disease developed to some extent in the seedlings, especially on tomato and eggplant and to a lesser extent on pepper. The seed was

* Formerly Assistant Plant Pathologist in the Insular Experiment Station of Porto Rico. Now in the Graduate School at Cornell University, Ithaca, N. Y.

sown in rows, four inches apart, in flats or beds. The soil used in these contained a large quantity of manure from a source known to be heavily infested with the wilt organism. As soon as any seedlings showed symptoms of the disease they were removed, a record being taken of the number of seedlings destroyed.

Seedlings were pulled from the seedbed when small, and planted in flats at a distance of four inches each way. Here they remained until large enough for transplanting in the field. The plants were removed with a ball of earth, reducing any temporary check in development as is the case when seedlings are transplanted without soil about the roots. A second advantage is that the seedlings suffer less and do not have the tendency to spindling growth, if for any reason transplanting is delayed. The less crowded condition helps in producing a hardier, stronger plant. A third advantage is the fewer chances of loss of seedlings during transplanting. It has been our experience that the mole-cricket attacks but few of the seedlings when they are transplanted according to this method.

The number of plants of each variety grown varied with the crop. The varieties were planted successively using four replications to reduce error.

Final counts were taken at the time when the second picking of fruit was made. Counts of diseased individuals in the seedbed and in the field are recorded together.

The results of incidence of the disease are given in the following tables.

TOMATO

TABLE 1.

INCIDENCE OF DISEASE IN COMMERCIAL VARIETIES OF TOMATOES, FIELD PLANTING

Variety	Population	Healthy	Diseased	% Diseased
Ponderosa	200	51	149	74.5
Pear Shaped Yellow	200	74	126	63.0
Gulf States	200	76	124	62
Louisiana Pink	200	79	121	60.5
Payne's Victory	200	83	117	58.5
Alameda Trophy	200	85	115	57.5
Early Detroit	200	89	111	55.5
Avon Early	200	91	109	54.5
Red Rock	200	94	106	53
San José Canner	200	94	106	53
Dwarf Champion	200	95	105	52.5
Maule's Matchless	200	102	98	49
Morse's Globe	200	102	98	49
Delaware Beauty	200	103	97	48.5
Bonny Best Early (Maule's)	200	104	96	48
Morse's No. 493, Special Early	200	109	91	45.5
Maule's Success	200	112	88	44
Marvana (U. S. D. A.)	300	188	112	37.33
Norton Wilt Resistant	300	189	111	37.0
Morse's Norton	300	192	108	36.0
Marvelosa (U. S. D. A.)	300	203	97	32.33
Morse's Marglobe	300	210	90	30.0
Stoke's Marglobe	300	218	82	27.33

From the above table it is clear that most commercial varieties of tomatoes are very susceptible to bacterial wilt. Ponderosa proved to be the most susceptible, almost three fourths of the population succumbing to the disease. Next in susceptibility is the Pear-Shaped Yellow (63 per cent) and the Gulf States (62 per cent). In a group ranging in susceptibility from 50 to 60 per cent, there are eight varieties. In a similar group from 40 to 50 per cent susceptibility, appear to fall six other varieties and between 30 and 40 per cent diseased plants there are four varieties. The least susceptible of the varieties were, in order of resistance: Marglobe (U. S. D. A.) (Plate XXXVI) with 23 per cent diseased plants; Marglobe (Stoke's) with 27.33 per cent, and Marglobe (Morse's) with 30 per cent diseased individuals. From the grower's standpoint a variety with a susceptibility higher than 30 per cent should not be recommended. It appears, that perhaps the Marglobe is the only tomato which may be grown with a reasonable degree of safety. It happens to be a good type, and the preliminary trials with it on the Island have shown it to be a good marketable tomato. Another tomato which proved to be somewhat resistant (32.33 per cent diseased) is the Marvelosa, distributed by the United States Department of Agriculture. It should be tested further.

A resistance test was also made of eight non-commercial Porto Rican varieties in order to locate resistant plants for breeding with varieties commercially desirable. Each one of these varieties was given a number in our records. They may be described briefly as follows: No. 1, small, about ½ inch–¾ inch in diameter, round; No. 2, slightly larger than No. 1; No. 3, pear-shaped, with longitudinal undulations, about 2 inches–3 inches long; No. 4, more or less oblong, about 2 inches × 1½ inches; No. 5, typically pear-shaped, small; No. 6, flat, large, so-called "platillo" (plate-like), much wrinkled; No. 7, flat, small, smooth; No. 8, flat and smooth, intermediate in size between No. 6 and No. 7.

Two hundred plants of each kind were grown. The symptoms of the disease appeared shortly after transplanting. The final data were collected in July, 1927. The results show that all plants died in all the strains except No. 6 in which 78.5 per cent were diseased.

It is evident that none of the native tomatoes are resistant and, therefore, are not to be used as material for breeding for disease resistance.

PEPPER

The results of tests with commercial varieties of pepper are given in table 2.

TABLE 2.

INCIDENCE OF DISEASE IN ELEVEN COMMERCIAL VARIETIES OF PEPPER.

Variety	Population	Healthy	Diseased	% Diseased
Early Giant	184	135	49	26.63
Ruby Giant	172	129	43	25.00
Tomato Salad	192	146	46	23.96
Large Bell	188	143	45	23.92
Neapolitan	185	143	42	22.70
Improved Ruby King	188	149	39	20.74
Crimson Giant	183	146	37	20.22
Ruby King	178	142	36	20.20
Chinese Giant	191	155	36	18.85
Worldbeater	189	154	35	18.52
Bull Nose Pepper	162	134	28	17.28

It will be seen that the populations are not uniform for the varieties, although 200 plants of each variety were set in the field. The reduction is due to injury by the mole-cricket to the seedlings which were not very large at the time of transplanting.

From the results given in the above table, it may be inferred that only Early Giant and Ruby Giant were heavily affected. However, the differences in susceptibility among the remaining varieties are not very significant. Three varieties Bull Nose, Worldbeater and Chinese Giant appear to have greater resistance than the others. Ruby King, Crimson Giant and Improved Ruby King are almost equally resistant.

Sixteen different forms of Porto Rican pepper were collected from various places in the Island and tested during the summer of 1927 in the same way as the imported commercial varieties.

Briefly described these forms are as follows:[1] No. 1, flat, wrinkled, hot pepper, about 1 inch in diameter; No. 2, hot, ovoid, 2 inches-2½ inches long, pointed at the blossom end; No. 3, long, large, hot, with a few longitudinal undulations; No. 4, "ají caballero", very small, hot, more or less conical and usually known as *Capsicum Baccatum;* No. 5, long, narrow, sweet, smooth, ends pointed, light green color; No. 6, Bull Nose type; No. 7, long, rather broad, angled, with blunt tip, about 6 inches long, light green color, sweet, so-called "Mallorquín"; No. 8, large, short, broad, almost as long as it is broad, dark green color, resembling the imported Chinese

[1] Descriptions for Nos. 10, 11, 12, and 14 are omitted. These forms were all nearly alike and similar to No. 9.

Giant, sweet; No. 9, a peculiar hot pepper with narrowed ends, broadened at about the lower third and pointed at the blossom end, purple toward the peduncle when not ripe, long peduncled, red when mature, so-called "ají platero" in Coamo; No. 14, a medium-sized sweet pepper with blunt tip, light green color; No. 15, rather long and broad, smooth, pointed abruptly at the blossom end, ratio of length to width 2:1, sweet; No. 16, extra long, sylindrical, hot pepper, so-called "ají picante" in Cayey.

One-hundred plants of each form were tested for resistance and the results are given below in the order of susceptibility: No. 6, 29 per cent diseased; No. 8, 27 per cent; No. 10, 23 per cent; No. 5, 21 per cent; No. 7, 21 per cent; No. 2, 19 per cent; No. 14, 18 per cent; No. 1, 17 per cent; No. 13, 17 per cent; No. 3, 16 per cent; No. 9, 16 per cent; No. 12, 16 per cent; No. 15, 16 per cent; No. 11, 15 per cent; No. 4, 12 per cent; No. 16, 12 per cent.

It is evident that the forms Nos. 6 and 8 are even more susceptible than the imported varieties given in table 2. These are, unfortunately, the two most desirable types in shape and size and the ones which would, therefore, offer the most suitable material for breeding work. The most resistant of the native forms are the hot peppers Nos. 4 and 16, both of which showed 12 per cent infection. A group with percentages of infection ranging from 15 to 19 includes forms Nos. 11, 3, 9, 12, 15, 1, 13, 14 and 2. These are given in order of relative resistance. Of the sweet peppers in the group the best in size and shape are Nos. 14 and 15. The remaining three forms, Nos. 5, 7, and 10 are undesirable; the first and third because of their shape and size and the second,—"mallorquín", because of its very light color and tender skin which does not stand much handling.

In general, it may be said that the hot peppers showed much higher resistance than imported sweet peppers, and that native forms of sweet pepper were not significantly higher in resistance than the imported varieties.

EGGPLANT

The following varieties of eggplant have been tested during the four-season period: six imported and five from the Island. Table 3, shows the relative susceptibility of each variety. The forms from Porto Rico are given in bold-face type.

TABLE 3.

INCIDENCE OF DISEASE IN IMPORTED AND PORTO RICAN VARIETIES OF EGGPLANT.

Variety	Population	Healthy	Diseased	% Infection
Black Beauty	500	72	428	85.60
Excelsior	500	85	415	83.00
New York Spineless	250	44	206	82.40
Large Round Purple	250	45	205	82.00
New Orleans Market	250	57	193	77.20
University Pink	250	69	181	72.40
Florida High Bush	500	152	348	69.06
Pompadour Purple Striped White	250	81	169	67.60
Long Purple	250	118	132	52.80
Camuy	500	444	56	11.20
Long Green	500	484	16	**3.20**

It is very evident that imported varieties of eggplant are extremely susceptible to the bacterial wilt and that, therefore, they can not be planted in infested soils without considerable risk. The varieties from Porto Rico are very resistant with the exception of "Pompadour" and University Pink. It is very unfortunate, however, that these resistant varieties do not possess the qualities of a commercial variety. The Long Green (Plate XXXVII) for instance, the most resistant of all (only 3.20 per cent infection) is a long, gourd-like green fruit and the "Camuy" (11.20 per cent infection), is a small, cylindrical purple fruit. The Long Purple, another form from the Island is of similar shape as the long green, but as will be seen from the table, it is quite susceptible to the disease.

The most susceptible variety was found to be the Black Beauty (Plate XXXVIII) with 85 per cent infection, followed closely by the Excelsior (83 per cent), New York Spineless (83.40 per cent), the Large Round Purple (82 per cent) and the New Orleans Market (77.20 per cent). Of the imported varieties, the most resistant appeared to be the Florida High Bush with 69.06 per cent infected plants, but it is doubtful if this variety can be successfully grown in infested lands on a commercial scale.

POTATO

Four varieties of potatoes of those which are being tested in the Island were planted in order to find out whether they were susceptible to the wilt. These were as follows: Bliss Triumph, Irish Cobbler, Green Mountain, and Spaulding Rose.

Twenty-five hills of each were planted in the field where the tests with eggplant, pepper, and tomato were conducted. The disease appeared on all varieties three weeks after planting and all plants were

infected by the sixth week. This experiment, although not dealing with a large population, indicates the high susceptibility of these varieties to the wilt, so that these potatoes should be planted here with this fact in mind. Infested fields should not be planted to potatoes.

TOBACCO

The following varieties of tobacco have been planted in infested land: "Virginia Blanco", the standard Porto Rican filler; "Ceniza"; "Vuelta Abajo"; a mammonth type labeled Station; Consolation (Yellow); Porto Rico Wrapper No. 1; "País"; Turkish; and two forms imported from Colombia, South America. Not a single case of the disease has appeared in any of the varieties. Wounding of the roots was practiced in order to increase the chances for the entrance of the causal agent. This was done by digging a number of plants of each variety when they were about a foot in height. The roots were then severely pruned and injured and the plants set again in their places. No cases appeared even with this treatment. It should be noted that in these experiments the susceptible Black Beauty eggplant was used as a check.

Another scheme was followed. Black Beauty eggplants which showed unquestionable symptoms of the wilt were dug out and tobacco plants set in their place. Even in this case the tobacco plants failed to become affected with the disease.

One hundred tobacco seedlings in pots and an equal number of plants about one foot in height, also in pots, were inoculated on roots, stems, and leaves. The cultures employed were isolated from eggplants and tomato plants. Six plants from the first lot became badly wilted, but a study of the lesions and a microscopic examination of them showed this wilting to have been caused by *Phytophthora Nicotianae*. All the remaining plants developed normally to maturity, bore seed, and when finally examined revealed no traces of injury by the inoculum.

From the above it seems that this bacterial wilt does not affect tobacco in Porto Rico.

INFECTION OF NORMALLY RESISTANT EGGPLANT VARIETIES

To ascertain whether normally resistant varieties of eggplant will contract the disease if artificially inoculated, a set of fifty plants of the Long Green variety was inoculated in 1928 with masses of bacteria oozing out of the vascular bundles of diseased Black Beauty

eggplants. Inoculation was made on tender twigs and on the woody stems by lifting the bark, injuring the woody tissues and transferring the inoculum into these wounds. Twenty-two of the fifty plants took the disease but it did not become generalized. From this experiment it appears that the disease may be induced on a normally resistant variety of eggplant through inoculation. The Long Green variety was shown in table 4 with a percentage of infection of only 3.2 while here 44 per cent have been infected.

RECOVERY OF DISEASED EGGPLANT

Recovery of diseased plants of resistant eggplant varieties has been found to occur. In the season 1929–30 it was found, for the first time, that a few plants of the Long Green variety which had been labeled as wilted or wilting had completely recovered. A closer inspection of the field was made and all plants that showed symptoms of the wilt were tagged on January 3, 1930. Bi-weekly observations were made recording the new cases of wilt or cases of recovery. The data collected up to March 9th. is as follows: out of 32 wilted plants, seven recovered and 25 died, a recovery of 21.89 per cent. Whether this may be expected in a larger population cannot be said. Similar counts with diseased Black Beauty eggplants were made and it was found that none of the wilted plants recovered. This would indicate that recovery takes place only in the resistant varieties.

GENERIC AND SPECIFIC SUSCEPT REACTIONS

It has been shown in the foregoing that the bacterial wilt of some solanaceous crops in Porto Rico severely affects eggplant (*Solanum Melongena* L.), pepper (*Capsicum baccatum* L., potato (*S. tuberosum* L.) and tomato (*Lycopersicon Lycopersicon* (L.) Karst.). The higher susceptibility of potatoes, tomatoes, and eggplant as compared to peppers is very clear from the data furnished in the preceding paragraphs.

Wilted zinnias (*Crassina Elegans* (Jacq.) Kuntze), were examined and an organism similar to or identical with that obtained from the eggplant, potato, and tomato, was isolated from them. A few cases of the disease on *Solanum torvum* L., a common weed, have been observed in the Station plots. The organism isolated from it is indistinguishable from that obtained from the aforementioned suscepts. The disease, however, is not very common on this suscept. The disease also occurs on the deadly night-shade (*Solanum Nigrum* L.).

Seaver and Chardón (**6**) list a wilt disease of solanaceous crops caused by *Bacterium solanacearum* E. F. Sm. and they evidently had in mind the same disease with which we have been dealing. *Solanum Wrightii* Benth., a recent importation from Panamá, has been repeatedly inoculated without success. Tobacco (*Nicotiana Tabacum* L.) has always failed to take the disease either through inoculation or under natural conditions. This is of particular interest in these studies because what is held to be the same disease occurs on tobacco in the United States where it is known as the Granville wilt. The same or a similar wilt is also found in many other tobacco countries.

Smith (l. c.) states that he received a report from Mr. Iorns, of Porto Rico, in which he held that *Solanum Mammosum* is resistant to what was, evidently, the disease under consideration.

The disease, then, appears to affect seven species of the higher plants, in four different genera from two families, the Solanaceae and the Carduaceae.

SYMPTONS

MORPHOLOGIC SYMTOMS

On the eggplant.—The first symptoms of the disease on eggplants are evident as a slight yellowing of the tender leaves of one or several shoots, followed by a severe wilting of these parts. The symptoms may appear on one branch alone or on several or may involve the whole plant. The period elapsing between the appearance of the first symptoms and the complete death of the suscept is usually from 7–10 days in the case of the very susceptible varieties like the Black Beauty, but it may be two weeks or longer in resistant varieties. In rapidly growing plants with abundant tender vegetative parts, the symptoms may have the appearance of lesions produced by scalding with boiling water or scorched by intense sun light.

On resistant varieties the symptoms may appear on one branch only which may be gradually killed while the rest of the plant remains healthy for a long time. Very frequently this is also affected. This is a very peculiar behavior of the eggplant, similar cases not being observed on any of the other suscepts. As discussed elsewhere in this paper a large proportion of the affected individuals of resistant varieties may recover from the disease.

In advanced cases the symptoms are those of the necrotic type, especially during rainy weather, when the tissues are overrun with

saprophytes. The leaves may show a rotting which invades the petiole and later the stem.

When plants are pulled and examined it is found that a large number of the roots have rotted, and upon lifting the cortex, a dirty white to creamy exudation, swarming with bacteria, is discovered.

On tomatoes.—The symptoms on tomatoes appear as a sudden wilting of the twigs and branches as in the eggplant. They resemble wilting produced by a cutting of the vascular bundles at some place below the wilted portion. They also resemble the condition resulting from attack by some sucking insects. The disease seems to start more frequently in the roots, which are much affected, and from there the organism works up the conducting system into the vegetative parts. Incipient roots are extensively developed on the stems of affected plants. It should be noted that this symptom has been reported for the same, or a similar disease, which occurs in other countries. Another symptom often observed, is a swollen condition of the stems. This has been reported by Smith (**8**) and others.

In the year 1927, during a very rainy season, some tomato plants showed a browning and rotting of the petioles of wilted leaves. Cankers were evident at the axils of the leaves. Isolations of a bacterium were made from the cankers and upon injection into healthy tomatoes, the characteristic wilting was produced, thus linking this symptom with the wilt. It is thought that the cankers were a result of the continuous rainy weather which helped to create a condition in the plant whereby they responded differently toward the organism. These symptoms were not repeated by inoculation, nor were they encountered in the succeeding seasons.

On peppers.—On this suscept the disease is characterized by a yellowing of the leaves at the margins. The discoloration may only include a few leaves, before the complete wilting of the plant occurs. Rolling or curling up of the yellowed leaves may occur, a symptom which has not been found on any other suscept. Peppers appear to be able to live longer than eggplant, tomato, or potato after the appearance of the first symptoms. Affected plants may gradually become defoliated before they finally succumb to infection.

On potatoes.—On the potato the disease is characterized by a yellowing of the leaves of young or immature shoots, or a sudden withering and death of stems and leaves. The disease spreads very rapidly in potato plants, one after the other succumbing to its ef-

fects. The symptoms in advanced stages are similar to those of plants which are undergoing a natural death.

In general, it may be said that the symptoms appear on the underground and all above-ground parts except the flowers and fruits.

HISTOLOGIC SYMPTOMS

Stems of all the suscepts show a browning of the vascular bundles which may extend from the roots to the petiole and main veins of the leaves. This browning of these tissues is very characteristic and is used in diagnosing the disease in those suscepts where morphologic symptoms are not very clearly defined. When infection begins on the leaves, the browning works down the veins into the midrib, thence down the petiole and into the stem. The bacteria which cause the disease are found massed in the vascular bundles and in great abundance in the stem in the region near the surface of the soil and in the roots. When either the affected roots or stems are cut transversely, the bacteria will ooze out in a short time in the form of a dirty white to creamy exudation.

In advanced stages the pith is also involved. In tomatoes and potatoes it soon breaks down into a soft mass; while in the eggplant and pepper it is slowly decomposed, the stems retaining their rigidity and finally becoming hollow.

In potatoes the pathogen extends into the tuber, resulting in a browning of the bundles there as in the stems. Thus, when an affected tuber is cut transversely, a browned ring will be evident underneath the superficial cork layer. In large tubers which have recently contracted the infection the vascular ring is browned only a short distance beyond the stem end. If these are kept for a few days, however, the browning extends to the opposite ends and in still later stages a rotting of the tubers from the ring inwards with complete destruction is reached in ten to fourteen days.

ETIOLOGY

IDENTITY OF THE PATHOGEN

The identity of the pathogen causing the bacterial wilt of some solanaceous crops in Porto Rico has not been definitely established, although it has been referred to as *Phytomonas solanaceara* (E. F. S.) Com. S. A. B. (*Bacterium solanacearum* E. F. Sm), by Henricksen (**3**), Stevenson and Rose (**9**), Stevenson (**10**), Thomas (**11**), Ló-

pez (**5**), and Seaver and Chardón (**6**). It is interesting to observe that Smith (**8**) basing his opinion on tomatoes and eggplants received from this Island, regarded the disease identical with the brown rot of the Solanaceae which occurs in the United States and other countries. This is probably the first report of the disease in which the organism from Porto Rico was studied.

Suspicion that the pathogen might be an altogether different one was raised when Cook (**1**) reported that the organism causing a bacterial disease of eggplants at the Insular Experiment Station would infect tomatoes and peppers, but not tobacco, and also by the fact that the author also failed to get infection with it on tobacco.

The organism was isolated from various suscepts, cultural studies made, and cross-inoculations performed in an effort to further elucidate the problem.

CULTURE CHARACTERS

On nutrient glucose agar: A dirty white colony which produces a brown discoloration at the end of four to five days.

On steamed potatoes: A gray white colony producing pronounced browning.

On potato cylinders: A dirty white growth, staining the potato brown and causing a slow rot. The rotted portion becomes browned first and finally blackened.

On glycerin agar: Colonies dirty creamy to dirty white with only very slight browning.

On Litmus milk: Litmus is slightly reduced and curdling does not set in.

On milk: No curdling produced.

On nutrient bouillon (plus peptone): browning occurs and there is also some precipitation.

On peptone water: No browning.

On Dunham's solution: Pnorounced clouding.

On lactose agar: Growth dirty white producing much browning after eight to ten days.

No gas production was observed in fermentation tubes in peptone water or bouillon with the following sugars: dextrose, maltose, lactose, dextrine, laevulose, or saccharose. There was no clouding in the column of medium at the closed end of the tubes which indicates an aerobic organism.

The behavior on the above media was alike for the following cultures:

No. 1 and 4	From eggplant	Experiment Station 1926
No. 6	From eggplant	Manatí 1926
No. 7 and 9	From tomato	Experiment Station 1926
No. 12	From tomato	Manatí 1926
No. 14	From zinnia	Experiment Station 1927
No. 18 and 19	From potato	Cayey 1927
No. 20 and 21	From eggplant	Cayey 1927
No. 24 and 27	From tomato	Experiment Station 1927
No. 28 and 30	From pepper	Experiment Station 1927
No. 33	From eggplant	Experiment Station 1928
No. 36	From *Solanum Torvum*	Experiment Station 1928
No. 37	From tomato	Experiment Station 1928
No. 38	From *Solanum Nigrum*	Experiment Station 1928

These cultures have been given in the order in which they were isolated. The irregularity in numbering is due to the fact that transfers were made and sub-cultures numbered before browning of the medium occurred and before the results of inoculations had been secured when a number of cultures were destroyed, thus accounting for the missing numbers in the series.

The organism proved to be gram negative.

From cultural characteristics alone it would seem that the organism that is responsible for the disease in eggplant, tomato, zinnia, potato, and pepper is probably the same.

PATHOGENICITY STUDIES

A large number of cross-inoculations have been made with the organism from the various sources. Considerable difficulty was experienced in this work since the organism appears to lose its virulence quickly in ordinary culture media and especially because the colonies from poured plates are similar during the first few days to those of non-pathogenic bacteria which also appear in the same plates.

The following scheme was at first adopted: plants were inoculated with the organism from the colony from which the subculture was made, usually five or six days after plating. The organism appeared to be virulent at this age. Later it was found that the organism retains the ability to infect its suscepts if it is carried in potato tubers or milk. The organism was most virulent when transferred directly from the affected parts to the plant to be inoculated. In this case the parts used for isolation were the bases of the stems, cut usually near the soil level. The portions of the stems were sterilized in mercuric chloride, after which the lower end was still further cut back with a sterile knife. These portions were then placed in sterile petri dishes, the bacterial ooze showing on the lower cut surface in a few minutes. Inoculations made with this ooze scarcely ever failed.

Almost invariably the inoculations were made on tender twigs or shoots at the axils of the leaves. It was only in the case of tobacco

that inoculations were made directly on the main stem, a few inches above the surface of the soil.

Infection through the roots was obtained merely by removing large plants from pots on which they had been growing, shaking out the soil around a few of the larger roots, wounding these and then planting in infested soil.

In every case the plants were cut after the wilt symptoms were evident and examined for browning of the vascular bundles, when the inoculations were pronounced as positive or negative.

The following varieties were used for inoculation Black Beauty: eggplant, "Mallorquín" and Ruby Giant peppers, Ponderosa tomato, and Porto Rico Filler tobacco. The potatoes were secured in the market and the variety is unknown.

The results are given in the following tables:

TABLE 4.
RESULTS OF INOCULATIONS AND CROSS-INOCULATIONS ON VARIOUS HOSTS—INOCULUM FRCM FRESH COLONIES

Culture		Tomato		Eggplant		Pepper		Potato		Tobacco	
No.	Source	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
1	Eggplant	6	0	6	0	4	2	5	1	0	6
2	Eggplant	0	4	0	4	0	4	0	4	0	4
3	Eggplant	0	4	0	4	0	4	0	4	0	4
4	Eggplant	4	0	4	0	4	0	4	0	0	4
5	Eggplant	0	4	0	4	0	4	0	4	0	4
6	Eggplant	4	0	4	0	3	1	4	0	0	4
7	Tomato	4	0	4	0	3	1	4	0	0	4
8	Tomato	0	4	0	4	0	4	0	4	0	4
9	Tomato	4	0	4	0	2	2	3	1	0	4
10	Tomato	0	4	0	4	0	4	0	4	0	4
11	Tomato	0	4	0	4	0	4	0	4	0	4
12	Tomato	4	0	4	0	3	1	4	0	0	4
13	Tomato	0	4	0	4	0	4	0	4	0	4
14	Zinnia	4	0	4	0	3	1	4	0	0	4
15	Zinnia	0	4	0	6	0	6	0	6	0	6
16	Zinnia	0	4	0	6	0	6	0	6	0	6
17	Zinnia	0	6	0	6	0	6	0	6	0	6
18	Potato	6	0	6	0	4	0	4	0	0	6
19	Potato	6	0	6	0	3	1	4	0	0	6
20	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
21	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
22	Eggplant	0	6	0	6	.....	.....	.....	.....	.....	.....
23	Eggplant	0	6	0	6	.....	.....	.....	.....	.....	.....
24	Eggplant	6	0	5	1	.....	.....	.....	.....	0	6
25	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
26	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
27	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
28	Pepper	6	0	6	0	6	0	6	0	0	6
29	Pepper	0	6	0	6	0	6	0	6	0	6
30	Pepper	6	0	6	0	5	1	6	0	0	6
31	Pepper	0	6	0	6	0	6	0	6	0	6
32	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
33	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
34	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
35	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
36	*Solanum Torvum*	6	0	6	0	6	0	.....	.....	0	6
37	Tomato	6	0	6	0	.....	.....	.....	.....	0	6
38	*Solanum Nigrum*	6	0	6	0	6	0	.....	.....	0	6
39	*Solanum Nigrum*	0	6	0	6	.....	.....	.....	.....	0	6

The results in table 4 show clearly that the pathogen in eggplant, pepper, potato, and tomato is identical. It is striking that in no

case did tobacco become infected, a total of 196 inoculations having failed.

TABLE 5.

RESULTS OF INOCULATIONS WITH OOZE FROM THE STEMS

Source	Eggplant		*Solanum Torvum*		*Solanum Wrightii*		*S. Nigrum*		Zinnia		Tobacco		Tomato	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Eggplant...	24	1	0	36	0	24	12	0	6	4	0	36	12	0
Tomato...	24	0	0	24	0	24	12	0	8	2	0	36	12	0

When the bacterial ooze, both from eggplant and tomato, was used as inoculum, infection was obtained on tomato, eggplant, *Solanum nigrum*, and on zinnia. Tobacco, *S. Torvum* and *S. Wrightii* did not become infected.

TABLE 6.

RESULTS OF INOCULATIONS ON ROOTS

	Eggplant		Tomato		Pepper		*Solanum Torvum*		Tobacco	
	+	−	+	−	+	−	+	−	+	−
Wounded	22	0	22	0	11	1	1	23	0	24
Not wounded	39	11	41	9	28	22	0	24	0	50

From the results in table 7 it may be concluded that invasion through wounded roots takes place readily in infested soils, hence the practical conclusion may be drawn, that these solanaceous crops should be carefully cultivated so that the danger from disturbing the roots may be minimized as much as possible. The amount of infection in the different suscepts was as much as could be expected from susceptible varieties in a heavily infested soil. It is significant that tobacco did not succumb to the disease even when roots were heavily wounded. *Solanum Torvum* did not take the disease, except in one case, a result which is in harmony with that in table 5. This behavior of this wild suscept from which an organism similar in every respect to that recovered from eggplant, tomato, and the other suscepts was obtained, is rather peculiar. That a few plants were once infected by the organism, as must have been the case in the few individuals in which the disease was recorded and from which the organism was isolated, may have been due to a certain set of predisposing conditions within or without the plant or to great virulence of the invading pathogen. This is as far as we are able to explain. It is obvious that further studies on this suscept should be made.

LIFE HISTORY

The pathogen seems to live over in soils for a long time. In the Experiment Station grounds it has existed in the soil for at least ten years. This estimate is based on reports that the author has had from former horticulturists.

Plant remains from previous crops are an important source of inoculum. Manure has been found to be a carrier of the pathogen which appears to be able to live in it. The manure at the Insular Station has been shown to be infested with the organism, hence the infestation of all soils fertilized with it. The infestation of manure was proven by the following experiment. Six tin cans (5-gallon capacity) were filled with well-rotted manure, another six with soil known to have produced healthy eggplants and still a third series with a 1–1 mixture of such soil and manure. Healthy Black Beauty plants about six inches high were transferred with a ball of earth around the roots (these were slightly injured) to these cans which were kept in the greenhouse. All the plants grew to normal development until the first flower bud appeared, when two of those in the cans with soil and manure and three in those with manure alone showed unmistakable wilt symptoms. Three weeks later all the plants in the cans with either manure alone or with the mixture of soil and manure had contracted the disease. The plants in the cans with soil alone remained healthy to maturity.

Invasion occurs under natural conditions through leaves and roots. In plants which have been beaten strongly by the wind the lower leaves are much injured by the constant friction against the soil and it is these leaves which are often the first to be infected. Injured roots also provide entrance for the pathogen. As far as we have been able to ascertain the pathogen is only capable of invading plants through wounds. In the inoculation experiments given in the preceding paragraphs it would seem that this is not the case, because when plants were not injured a large number of them succumbed to the attacks of the malady. But it should not be forgotten that the roots of all the suscepts employed in these experiments were always subject to a certain amount of injury by animal pests.

In the experiment with manure in cans, where these soil inhabitants were obviated, the pathogen, no doubt, gained entrance through the wounded roots. By reason of unsuitable equipment we failed to conduct an experiment where these soil pests could be included.

During the growing season of 1929–30 the green beetle (*Diabro-*

tica graminea Baly) swarmed on our eggplants following which the disease appeared to extend considerably over a large field. In order to determine whether this insect was at all responsible for the dissemination of the pathogen a series of experiments was begun, using wire cages. Six healthy plants (Black Beauty eggplant) were covered with these cages. At the end of ten days they were examined and found to be healthy. The insects (*Diabrotica*), which had been fed for six days with twigs of diseased plants on which the leaves had wilted and the brown stain was evident, were then transferred to these cages, ten to fifteen to each cage. The insects were kept in the cages for two weeks when the first case of wilting was found in one of the cages. A second case appeared four days after the first and two more plants showed the disease twenty days after the insects had been transferred to the cages. Two plants remained healthy throughout the season. Six plants, also covered with cages, but from which the beetle was excluded, did not show any wilt symptoms. It may be concluded that *D. graminea* is a disseminating agent of the disease. (Plate XXXIX).

EPIPHYTOLOGY

The pathogen seems to attack most frequently plants growing in low, wet places, but the disease also appears on higher, well-drained locations. From our observations it may be inferred that the malady is more prevalent in heavy soils; nearly all the cases reported to us have come from sections having heavy soils. The disease is very severe in Río Piedras and in the Cidra-Cayey district where the soils are predominantly of the heavy class. But, in the sandy loams of the intervening section of Caguas no cases have ever been found by the writer or reported by others.

The appearance of the disease is in no way related to the age of the suscepts. We are inclined to believe that it coincides with certain seasonal changes, as for example, periods of continued rains followed by short periods of dryness. In our plantings the first cases were observed during one such rainy period following a dry season.

CONTROL

General eradicatory measures are always helpful in reducing the foci for inoculum and consequently furnish important means of control. However, they do not go very far in preventing heavy losses to our solanaceous vegetable crops. The removal of diseased plants is to be recommended but it must not be overlooked that the causal

agent lives in the soil in which it is capable of existing for a long time. A practical method of soil treatment which will kill the organism is desirable. But such a measure does not seem likely to be developed with our present knowledge of the malady. Preventive measures appear to us the most logical solution for the problem.

BREEDING FOR DISEASE RESISTANCE

The preliminary trials with varieties of eggplant, pepper and tomato, have made it clear that breeding for resistance is not promising for the latter two crops, since native and imported commercial varieties were more or less equally susceptible to this disease. Consequently, development of resistant strains in these must be sought through selection within the commercial varieties. On the other hand, some native varieties of eggplant showed a very remarkable superiority in resistance over any of the imported varieties. Unfortunately their shape, size and color are not suitable for the market thus making it impractical to extend their cultivation for commercial ends but they are suitable for crossing with desirable commercial sorts to secure desirable resistant plants.

Breeding work on tomato and pepper was not started by the author because he was engaged on other problems. It is only on the eggplant that he has endeavored to develop resistant strains.

Several crosses were made in 1926 between the imported susceptible varieties of desirable shape and color and those of Porto Rico showing decided resistance to the malady. Much progress has been made in this line and some of the results will be reported on later. Several strains which combine the desirable characteristics of both parents in each case, have been isolated and are under trial.

It was even thought that strains might be developed from the susceptible types. With this purpose in mind two selections in the Black Beauty variety were made in 1927. One of these was discontinued in 1928. That year two other selections were made from the one retained. Although in 1929 these did not show any higher resistance than the plants raised from imported seed, yet two further selections were made. These were given a final test in 1929–30 when they were abandoned because of their high susceptibility.

GRAFTING ON RESISTANT STOCKS

Grafting the eggplant on *Solanum Torvum* has been suggested by Iorns (**4**) for the control of diseases. Eighteen grafts of the Black beauty eggplant on *Solanum Torvum* were made in 1929–30 of which

only eight lived. The stock had been planted in our most heavily infested plots. All the grafts developed excellently without showing the symptoms of the disease. Of these plants five were destroyed by heavy winds, breaking the stems at the junction of stock and scion. The three remaining plants grew to maturity, bearing many fruits. They were then inoculated with ooze from Black Beauty diseased plants and all contracted the disease. This shows that as long as infection depended on invasion through the root system the plants were safe; but when the inoculum was introduced into the stems of the scion the disease developed. This seems to indicate that the stock has no influence on scion in respect to resistance.

GENERAL CONTROL MEASURES

Certain control measures which are always helpful should be enumerated here: (*a*) proper drainage of the land, (*b*) careful cultivation to prevent root injury, (*c*) care in transplanting to injure the least number of roots, (*d*) avoiding the use of manure which has given indications of being infested with the pathogen, and (*e*) avoiding, when possible, the heavier soils.

DISCUSSION

A bacterial disease present in Porto Rico on some solanaceous crops was considered as identical to that of the Southern United States for the first time by Henricksen (**3**) who based his conclusion on the similarity of the morphologic symptoms of the Porto Rican disease and that of the continent. Later the pathogen was determined as *Bacterium solanacearum* E. F. S. by Smith (**8**) who had received some diseased plants from Mr. Iorns of the Porto Rico Agricultural Experiment Station at Mayagüez. The disease has been subsequently attributed to Smith's pathogen, but no experimental work has been done previously in Porto Rico to prove that assumption. Cook (**1**) in 1924 raised the question of the identity of the eggplant wilt organism which he believed would not infest tobacco. The author has found that the eggplant organism will not infect tobacco but the disease produced by it on the other solanaceous crops is evidently very similar. Still the doubt remains as to whether it is *B. solanacearum* which is responsible for the disease, since this organism causes a wilt of tobacco in other countries. The fact that the organism isolated in Porto Rico from the other suscepts has never infected tobacco leads to the conclusion that either tobacco is resistant, or that the organism is distinct from *B. Solanacearum* or is a strain of it unable to infect tobacco.

The symptoms of the disease described elsewhere agree in every respect with those of the Porto Rican malady. The description given for *Bacterium solanacearum* E. F. S. is essentially that for the pathogenic organism isolated from the various suscepts. The organism is gram negative; it develops as white to dirty white colonies which produce a brown stain on many media, especially on sugar media; it loses its virulence very readily in most culture media but retains it in potato tubers and in milk; it does not curdle milk or reduce litmus; does not cause the fermentation of sugars and is aerobic. Although the writer admits that much more extensive work should be done with the pathogen before its identity can be definitely established, he is inclined to regard it as a strain of *B. solanacearum*. The only reason why it should not be considered as identical is the fact that tobacco is not infected by it. We think that, in all probability, we are concerned with a strain different from that occurring on the continent. A second possibility is that all tobacco in Porto Rico is resistant to *B. solanacearum*. In the final elucidation of the problem the tobacco from Porto Rico should be tested in the Southern United States, for instance, or the varieties from the continent should be introduced and exposed here to the pathogen. To conclude, we tentatively assign to the Porto Rican organism the rank of a strain which is incapable of infecting Porto Rican tobacco. Since the virulence of the organism is maintained only with difficulty it did not seem possible to obtain cultures from abroad for use in inoculating our tobacco.

SUMMARY

1. A bacterial disease of solanaceous vegetable crops has been prevalent in Porto Rico for many years.

2. The disease affects tomato, potato, eggplant, and pepper; *Solanum torvum*, *S. nigrum* and zinnias become infected occasionally, while tobacco appears to be immune.

3. All imported varieties of tomato, pepper, and eggplant are more or less susceptible. The Marglobe and the Marvelosa tomatoes and the Bull Nose, World beater, Chinese Giant, Ruby King, Crimson Giant and Improved Ruby King peppers are very resistant. The most resistant of the imported eggplants is the New Orleans Market. The most susceptible of all suscepts used are the Ponderosa tomato, the Early Giant and Ruby Giant peppers, and the Black Beauty eggplant. Four varieties of potato were found susceptible.

4. Peppers are more resistant than either eggplants or tomatoes.

5. Native varieties of eggplant are more resistant than the im-

ported sorts; but all native peppers and tomatoes are very susceptible. Hot peppers proved to be more resistant than the sweet peppers.

6. The disease is very severe on tomatoes and to a less extent on eggplants, at the seedling stage.

7. Normally resistant varieties of eggplant may contract the disease if artificially inoculated.

8. Some diseased plants of the resistant eggplant recovered from the disease.

9. The symptoms of the disease are characterized as a yellowing and withering of the tender shoots and leaves and finally a complete wilting of the affected plant. Sometimes lesions having the appearance produced by scalding water or scorched by strong sunlight are evident. Cankers are formed occasionally in tomatoes. Histologic symptoms consist of a browning of the vascular bundles.

10. The pathogen is culturally indentical with *Phytomonas solanaceara* (E. F. S.) Com S. A. B. *bacterium solanacearum* E. F. S. or very similar to it. In cross-inoculation studies it is also similar to it, but is unable to infect tobacco. We are of the opinion that it is only a strain of that pathogen.

11. The organism loses it virulence very readily on most artificial culture media.

12. Successful inoculations with the pathogen in culture were obtained when the inoculum was used directly from the original isolation on the poured plate. Inoculations with the bacterial ooze obtained in more or less uncontaminated condition from the vascular bundles of diseased plants scarcely ever failed.

13. Natural infection occurs through wounds in the leaves and roots.

14. The pathogen may be disseminated by the green beetle, *Diabrotica graminea* Baly.

15. The pathogen seems to be harbored in manure, plant remains, etc., and appears to exist in soils for a number of years.

16. It is believed that the first appearance of the disease coincides with periods of continued rainfall followed by periods of drought.

17. The production of resistant strains of the various crops seems to offer a logical means of control. Breeding work in the eggplant is in progress.

18. Grafting of susceptible varieties on *Solanum Torvum* only eliminates the chances of root infection.

The writer wishes to express his gratitude to Hon. C. E. Chardón,

former Commissioner of Agriculture and Labor of Porto Rico and to Mr. F. A. López Domínguez, formerly Director of the Insular Experiment Station, who encouraged and supported the work on this problem. He is also greatly indebted to Dr. Mel. T. Cook, Plant Pathologist of the Insular Experiment Station, for his helpful advice and criticisms and to Professors H. H. Whetzel, M. F. Barrus and W. H. Burkholder for help in the preparation of the manuscript.

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Explanation of Plates

Plate XXXVI. A tolerant tomato plant at left and a susceptible (wilted) one at right.

Plate XXXVII. Upper. A susceptible variety of egg-plant.
Lower. A resistant variety of egg-plant.

Plate XXXVIII. An advanced case of wilt in the Black Beauty egg plant.

Plate XXXIX. At left—a wilted tomato plant.
At right—a cage used in the study of insect transmission.

PLATE XXXVI.

PLATE XXXVII.

PLATE XXXVIII.

PLATE XXXIX.

OBSERVATIONS ON THE BEAN LACE-BUG IN PORTO RICO

By MORTIMER D. LEONARD[1] and ALFRED S. MILLS[2]

SYSTEMATIC HISTORY

Corythucha gossypii was first described by Fabricius in Ent. Syst. 4, p. 78, 1794 as *Acanthia gossypii* from the island of Dominica in the West Indies. The species has been subsequently mentioned in systematic literature by Latreille, Burmeister, Herrich-Schaeffer and Fieber, all of whom placed it in the genus Tingis. Stal in 1869 referred it to the genus Galeatus but in 1873 placed it in the genus Corythucha where it has since remained.

DISTRIBUTION AND FOOD-PLANTS

The bean lace-bug has been recorded from Mexico, New Mexico, Texas, Florida, Central America, from Venezuela in South America and from the West Indies. In the latter it is the most common and widely distributed member of the genus Corythucha, feeding upon several different plants not closely related botanically. It has also been recorded from New York and New Jersey, but according to Barber and Weiss (5) and confirmed by Drake's extensive studies, it does not occur north of Florida. Records from these two states should probably be referred to *C. marmorata* Uhler.

There follows the detailed distribution data:

MEXICO.—In the United States National Museum this species is represented as follows: Specimens from the Uhler collection labelled "Mex."; several from Durango, Feb. 1918; one from Tampico, 1918; Aguas Calientes, one collected by E. A. Schwarz and many specimens collected by F. C. Bishopp, Dec. 1, 1909.

Ferris (16) found the insect in some numbers on castor beans (*Ricinus communis*) at San José del Cabo in Lower California and Dr. Drake also has material from Lower California. Van Duzee (36) records it from San Carlos Bay, State of Sonora, July 9, breeding in immense numbers on Palo San Juan; also on Coronados Islands, May 18, and on San Esteban Island, April 20. He states that the food-plant is *Atamisquaea emarginata.*

Dr. Alfons Dampf in a letter dated Feb. 7, 1931 to Dr. C. J. Drake states that this lacebug "appeared in enormous quantity on

[1] Chief Entomologist, Insular Experiment Station, Río Piedras, Porto Rico.
[2] Junior Plant Quarantine Inspector, United States Department of Agriculture.

castor beans (*Ricinus*) sucking the leaves and spoiling the harvest. According to the proprietor the bugs attacked nearly 60 hectares (150 acres) of this plant on the Hacienda El Palmar, between Jalapa and Vera Cruz, State of Veracruz, 700 meters above sea-level."

NEW MEXICO.—Dr. Drake writes that he has one specimen labeled "New Mexico". The United States National Museum has one specimen from the Uhler collection from castor oil without definite locality.

TEXAS.—Victoria, Dec. 1, 1909, F. C. Bishopp coll. and May 29, 1913 Mitchell and Coad coll. (United States National Museum.)

FLORIDA.—Recorded by Osborn and Drake (25) on *Ichthyometia (Piscidia) piscipula,* by Watson (37) as injurious to castor bean, and by Blatchley (6) from "Cape Sable and Marco, Feb. 20–Mar. 9, taken by sweeping Hibiscus and other plants along the margins of tidewater bayous. Recorded from Biscayne Bay and Miami." Dr. Drake writes us that he has specimens in his collection from Key West, Miami, Sebring, Gainesville, and Sanford. The United States National Museum has material from Silver Palm, May 15, 1923 (G. F. Moznette collector).

HONDURAS.—Drake (13) records it as extremely abundant near Tela and considerably injuring sour sop (T. H. Hubbell collector).

GUATEMALA.—The United States National Museum has 2 specimens from Pantaleon, 1,700 ft. Champion coll, which were recorded as *C. decens* in Biologia Cent. Am.

COSTA RICA.—In the United States National Museum are several specimens from *Prunus persica,* Dec. 19, 1913, without definite locality.

PANAMA.—In the United States National Museum many on Jatropha sp. from Bella Vista, Oct. 28, 1918 collected by Dietz and Zetek. Dr. Drake wrote under date of June 11, 1931 that he had recently received material from Mr. Zetek from the Canal Zone who stated that the insect was feeding on beans.

JAMAICA.—Gowdey (19) listed the insect as a pest of Congo pea (*Cajanus indicus*) and of Hibiscus. The United States National Museum has 2 specimens from Kingston. Van Duzee (33) lists it from Kingston, Mandeville and Montego Bay.

CUBA.—Drake (12) recorded several specimens having been taken at Guines, March 18, 1925, by Dr. J. G. Myers on castor bean. The United States National Museum has specimens taken on castor oil from Santiago de las Vegas (P. Cardin coll.) May 1918 and San Antonio de los Baños, April 1918. Bruner (7) records it as found

locally (Aug. 13, 1925) breeding on leaves of citron or cidra (*Citrus medica* L.) but not important as a pest of this plant.

HAITI.—Although the insect has apparently not heretofore been recorded from Haiti we have the following statement from Dr. H. L. Dozier, Chief Entomologist of the Department of Agriculture in Haiti under date of March 17, 1931 in which he says "it is common and very abundant, widely distributed everywhere in Haiti on beans and on 'bois cabrit' (*Cassia emarginata*)." Dr. Wm. A. Hoffman collected 1 specimen at Plaine Cul de Sac, Dumai, April 22, 1925 (United States National Museum).

SANTO DOMINGO.—Many specimens in the United States National Museum from La Romana Central, July 15, 1917, collected by H. Morrison.

PORTO RICO.—The insect is undoubtedly widely distributed in Porto Rico although it is possible that it is confined for the most part to the lower altitudes, not having been recorded as occurring higher than about 1,300 feet (at Cayey).

We have the following definite records mostly from notes in the Insular Experiment Station and from our own observations: in the north part of the Island from Pt. Cangrejos on sword bean (Wolcott coll.), 1912 and on beans 1925 (United States National Museum); Camuy, scarce on cotton, 1921; Río Piedras, on sword beans, (*Canavallia spp.*) March 1912, Wolcott coll. (United States National Museum); Ciales, on castor bean, 1913; Palo Seco on about two acres of pole lima beans and on several papayas during August, 1930; Río Piedras on lima bean, sour sop (*Anona muricata*) and on *Anona diversifolia* Safford, castor bean and papaya in 1930 and 1931; Mameyes on yautía, 1912; Luquillo on castor bean 1916; Dr. G. N. Wolcott found several young lemon and orange trees moderately infested in April 1931 at Isabela and the writers early in May made the same observation on young grapefruits at Río Piedras; material in the Drake collection also from Mayagüez; in the south part of the Island from Yauco, scarce on cotton, March 7, 1930; between Yauco and Guánica (Hacienda Santa Rita) on lima beans, badly infested August 21, 1930; Hacienda Santa Rita in Drake collection; Tallaboa, moderate on castor beans, August 21, 1930; Ponce, quite abundant on papaya, July 30, 1930; Guayama, on lima beans, 1917; and Cayey, (about the middle of the Island) quite abundant on papaya, August 2, 1930. We have also found the insect moderately abundant in April and July on castor bean on Vieques Island both at Puerto Real near the west end and at Salinas on the east end.

VIRGIN ISLANDS.—Wilson (40) reports the castor bean infested with this species on the islands of St. Croix, St. Thomas, and St. John. The same writer (41) also states that *C. gossypii* is a minor pest of cotton in St. Croix but seriously injures castor beans. We also found it abundant and very injurious in several garden patches of lima beans on the island of St. Thomas on August 11, 1930.

ANTIGUA.—Several specimens in the United States National Museum from castor oil plants on August 19.

MONSERRAT.—Ballou (1) stated that "a small patch of cotton was attacked by *Corythucha* the foliage of the plants being affected like that of castor oil plants attacked by the same insect." There seems to be no doubt that this refers to *C. gossypii*.

ST. VINCENT.—Watts (38) says "*Corythucha* has been noticed feeding on cotton leaves. This is apparently the same insect as that recorded from Monserrat." In Rept. Dept. Agr. St. Vincent for 1919 (39) it is stated that "the Corythucha bug was severe on castor oil plants grown at the experiment station during the year." Wilson (41) states it to be a minor pest of cotton. The United States National Museum has material collected by H. H. Smith on the "windward side."

GRENADA.—"Grenada" (Drake collection). There is also material in the United States National Museum collected by H. H. Smith from the Mount Gay Estate (leeward side.)

TRINIDAD.—Urich (32) records finding that a "Tingitid, *Corythucha* near *ciliata*, also found on the castor bean, seems to have a liking for dahlias, the leaves of which plant it soon causes to wither". Specimens are in the United States National Museum from castor bean from "Trinidad" collected by F. W. Urich, May 20, 1893 and several collected by W. E. Broadway, Oct. 14, 1908.

VENEZUELA.—Several specimens in the United States National Museum from Caracas collected by A. Ernst, Sept. 23, 1886.

Dr. Drake writes that he believes more collecting will greatly extend the distribution of this species in South America.

ECONOMIC IMPORTANCE

Judging from our own observations and records in literature this insect is by far more injurious to lima beans than to any other of its known food-plants. It has however, apparently not been recorded as attacking lima beans in any place but Porto Rico and the only previous mention of injury to this crop is by Wolcott at Guayama (1916) and by Smyth (1920). On August 11, 1930 a small

patch of lima beans at Charlotte Amalie in St. Thomas was observed to be badly infested. On August 21, 1930, we saw a small patch of lima beans at the Hacienda Santa Rita between Yauco and Guánica also badly infested. On August 29, 1930, we visited a grower at Palo Seco, across the bay from San Juan, who had over two acres of pole limas and string beans, the leaves of which were so badly damaged that practically no pods had formed, although the plants were all old enough to have been in full bearing. This grower stated that he had been troubled by the pest for several years and that it was more injurious during the summer, being the main reason why more lima beans were not grown during that season of the year. During the early part of September a severe infestation was observed on a fair sized garden patch of pole limas at Río Piedras on which pod formation had been almost entirely prevented. Two or three miles distant another younger planting which was just starting to blossom was beginning to show the start of an infestation.

It should be mentioned that in September, 1930 a bearing planting of bush limas at Cidra at an altitude of about 1,300 feet showed no infestation and the grower stated that although he had previously grown beans they had never been bothered by the pest.

As is well known the characteristic injury of lace-bugs is caused by the feeding of the nymphs and adults on the underside of the leaves from which they extract the juices. Where only a few insects are present a slight whitish discoloration of the upper surface is apparent, but as they become more numerous the whitish areas are greater in extent until in severe cases the whole upper surface of the leaf is involved. In some cases the entire surface may be mottled with darker spots and if sufficient feeding has taken place the leaves finally turn brown, wither, and fall off. The under surface becomes spotted and discolored with excrement (Plates XL and XLI). We have noted cases of such severe injury only to lima beans and to the four trees of *Anona diversifolia* on the Insular Experiment Station grounds mentioned above.

These Anona trees, about five years old, were introduced from Guatemala when about a year old and are about 8–12 feet high. By October and November 1930 the leaves were severely injured and many had dried up and fallen to the ground. By March 1931 these trees had set a new crop of healthy leaves and the lace bugs were scarce. In the case of beans the size and quality of the pods has been reduced or their formation almost entirely prevented. Injury that we have observed to other plants such as cotton, papaya, sour

sop and citrus has been confined mostly to comparatively small whitish areas on the upper surface of the leaves, altho the grower in Palo Seco where the severe injury to lima beans was observed, stated that twice in the past several years he had experienced considerable injury to his papayas.

LIFE HISTORY AND HABITS

The bean lace-bug may be found breeding on its various food-plants at all times of the year in Porto Rico. Breeding is continuous and rapid and although the exact length of time needed to complete a generation has not as yet been definitely determined it is apparently not more than about three weeks.

The eggs are laid on the underside of the leaves and are partially inserted into the leaf tissue usually along the midrib but are sometimes laid along the larger veins. They are often deposited within the tissue of the midrib or of the veins. As usual they are surrounded by a mass of black gummy substance which leaves only the cap slightly projecting or which may sometimes almost entirely cover the cap, but frequently eggs may be found projecting a considerable part of their length. A great many eggs may be laid upon a single leaf.

The nymphs usually feed in fairly compact clusters frequently in company with one or more adults. Both nymphs and adults are readily disturbed and the colonies tend to scatter when the leaf is turned over for examination.

DESCRIPTION OF STAGES

THE EGG.—(Fig. 1.) Length, .33 mm., greatest width, .15 mm.; flask-shaped with the neck bent somewhat to one side; anterior end obliquely truncate and provided with an oval lid which has a submarginal ridge enclosing a somewhat depressed area; the posterior end rounded; the chorion without sculpture, smooth and shining, the posterior half whitish, the anterior half blackish with the line between the two colors sharply defined

Fig. 1.—Egg greatly enlarged.

FIRST STAGE.—Length .4 mm.; upon hatching from the egg the nymph is at first pale greenish but the abdomen soon becomes somewhat darker green due to ingested food and the head and thorax become slightly tinged with brownish; body noticeably cylindrical as compared with the more flattened shape of the

later stages; antennae only about one third the length of the body and three segmented, last segment with several prominent hairs especially at the tip; eyes consisting of five prominent bright, red ommatidia; a median pair of moderately long black hairs near the front and a pair of longer black hairs on either side on the vertex between the eyes and the median line, each of the latter pair arising from a prominent tubercle; thorax only a little wider than head and with sides almost parallel, slightly brownish along the lateral margin. Pro- and meso-thoracic segments and abdominal segments two to nine with a dark lateral hair and in addition on the meso-thorax there is a median pair; abdominal segments five and six each with a pair of prominent black hairs either side of the median line arranged so as to form a transverse row, segment eight with a pair of median hairs somewhat shorter, each arising from a rather well-defined tubercle and just in front and inside of these, two somewhat shorter black hairs; legs pale yellowish, beak concolorous, with extreme tip distinctly blacker.

SECOND STAGE.—Length .6 mm.; body somewhat more flattened than in first stage and abdomen somewhat more explanate, coloring about the same as in the first stage except that segments four to six of the abdomen contain a little brownish pigment; head with two pairs of simple anterior hairs present in first stage now shorter and arising from an elongated protuberance, the anterior pair of hairs on the vertex also now arises from a prominent blackened conical protuberance, the other pair remaining simple and arising just caudad at their base; thorax distinctly wider than head; the two pairs of lateral hairs on the meso- and meta-thorax are now also much shorter and arise from a darkened conical protuberance; the meso-thorax has a pair of darkened conical protuberances ending in a short hair and from the base of each arise two simple hairs; the lateral margin of abdominal segments two to nine each with a pale prominent conical protuberance ending in a fine black hair and with a fine black hair arising on the base of each protuberance on abdominal segments two to seven; the two pairs of median black hairs on segments five to six remain the same as in the first stage except that they are shorter but the posterior pair on segment eight is now elongated into two conical protuberances.

THIRD STAGE.—Length .85 mm.; body considerably more flattened than in preceding stage with the lateral margin of the thorax and abdomen distinctly upturned; head, lateral margins of thorax broadly, and disc of abdominal segments four to six more distinctly

brownish than in preceding stage; conical protuberances on head considerably larger than in preceding stage, dark brownish and the posterior pair of the two cephalic hairs now both arising from a single base which is considerably elevated; thorax with the lateral conical protuberances of the pro- and meso-thorax more prominent than in preceding stage especially those on the meso-thorax; just in front of each is now a small setigerous tubercle and near the base of each two small black hairs; there is a median pair of small hairs on the pro-thorax; abdominal protuberances and hairs appear to be about the same as in the preceding stage except that the second segment now has a pair of pale conical protuberances.

FOURTH STAGE.—Length 1 mm.; number of ommatidia considerably increased, wing pads now distinctly formed, dark brownish, and reaching almost to second abdominal segment; antennae still seem to be but three-segmented; conical protuberances the same as before but those especially on the head and thorax proportionately larger; there are now two pairs of small median hairs on the pro-thorax; general color of body tending to be a little darker yellow with the brown of the pro-thorax confined to a subquadrate patch on either side of the median line in front and a transverse patch just in front of each wing-pad; the abdomen is now distinctly darkened toward the tip and the antennae are slightly infuscated toward the tip.

FIFTH STAGE.—Length 1.5–1.7 mm.; differs chiefly from the preceding stage in the following particulars: eyes have more ommatidia, pro-thorax wider and produced behind into a rounded point on the median line; wing-pads reaching backwards to the fifth abdominal segment and with a prominent lateral angle towards the front; armature on each caudo-lateral angle of pro-thorax consists of two spines, one longer than the other, and a simple hair all arising from a single tubercle; the lateral border of the wing-pads near the middle now has two prominent conical protuberances and a simple hair arising from a common tubercle, second abdominal segment with a pair of median whitish spines tipped with a short fine black hair; the brownish coloring is approximately the same as before on the head, pro-thorax, and abdomen but only the tip and base of the wing-pads are now darkened; antennae now four-segmented.

ADULT.—(The following description has been prepared for us by Dr. Carl J. Drake.) Size variable, usually about 3 mm. long and 1.6 mm. wide. The pronotum pale brown; reticulations white

and marked with brown or fuscous; areolae hyaline. Hood moderately large, slightly contricted behind the middle, rather closely reticulated, more or less marked with brown or fuscous, its length equal to one-half its width. Median carina strongly foliaceous, distinctly arched, subequal to length of hood, mostly biseriate, with a large brown or fuscous spot; lateral carinae rather short, small, raised anteriorly, terminating at base of triangular projection of pronotum, composed of one triangular areola. Antennae testaceous, segments III and IV with numerous long bristly hairs. Bucculae open in front. Mesosternum strongly sulcate, the rostrum extending behind the intermediate coxae.

Body beneath brown to fuscous; legs testaceous. Elytra with sides slightly concave, with distinct tumid elevation, the areolae somewhat variable in size; markings variable, sometimes almost wanting, usually forming two or three interrupted transverse brown to fuscous streaks. Wings a little longer than abdomen. Male claspers strongly curved, slender.

NATURAL ENEMIES

Although it would seem that the cap of the eggs is sufficiently exposed in most cases to permit of the introduction of parasite eggs, we have reared no egg-parasites nor have any other workers done so as far as we have been able to determine.

The exposed habit of the nymphs should render them an easy target for some of the smaller hymenopterous parasites but apparently this stage is also free from attack.

It is very interesting to note, however, that the larva of a gall midge (Cecidomyidae) has been recorded as attacking a lace-bug nymph in Portugal. This is referred to by H. F. Barnes in a paper entitled "Gall midges (Cecidomyidae) as enemies of the Tingidae, Psylidae, Aleyrodidae and Coccidae" in Bul. Ent. Res. **21** (3): 319, 1930. The brief reference in full is as follows:

Endopsylla endogena (Kieffer).
Kieffer, Zeits. f. Hymen. u. Dipt. 1, 1907, p. 129–130 (*Cecidomyia*); Genera Insectorum, fasc. 152, 1913, p. 222 (*Endopsylla*).

Male and pupa described. Kieffer bred a single male from a dead larva of *Stephanitis pyri F.* Pupation took place inside the Tingid and the adult emerged through a circular hole in the back of the host. The insects were sent by Tavares from Portugal. This species seems to be the most specialized gall midge

yet known being the only one known to live as an internal parasite and pupate inside its host.

We have found a small black and yellow spider on a considerable number of the leaves of the four trees of *Anona diversifolia* mentioned above as being badly infested with *C. gossypii* on the Insular Experiment Station grounds at Río Piedras. This spider has been determined by Prof. C. R. Crosby as *Theridula opulenta* Walckenaer, a cosmopolitan species and according to Petrunkevitch (Trans. Conn. Acad. Sci. **30**: 189–191, 1930) very common in Porto Rico. It spins a small web on the leaf and in several cases nymphs or adults of the lace-bug have been found caught within it. This spider however is probably not a very important factor in natural control.

A number of adults and nymphs, as well as of the brownish egg-masses of a Reduviid bug, *Zelus nugax* Stahl, have also been found on the leaves of the same trees. In several instances the nymphs have been observed feeding on those of the lace-bug. This Reduviid has been reported from several localities on the Island as having been found on various plants and trees but no observations have previously been made as to the insects upon which it is predaceous.

CONTROL

Corythucha gossypii has apparently never been sufficiently abundant on its various economic food-plants to make remedial measures seem worth while except in the case of lima beans.

When we first encountered the severe infestation on lima beans at Palo Seco, mentioned above, the grower told us that he had repeatedly sprayed with nicotine sulphate and soap at standard strengths but with poor success. He also mentioned that he had had equally poor results with the same combination in the control of the egg-plant lace bug, *Corythaica monacha* Stahl. A review of the literature dealing with definite experiments on the control of lace-bugs on various plants in various places disclosed the fact that nicotine sulphate was comparatively ineffective even when a small quantity of soap was added as a sticker and spreader. Crosby and Hadley, (Jour. Econ. Ent. 8: 409–414, 1915), although they did not use nicotine, state that the rhododendron lace-bug, *Stephanitis rhododendri* Horvath, (therein discussed under the name of *Leptobyrsa explanata* Heid.) was found to be readily controlled by the use of a soap solution, 1 pound in 10 gallons of water. Fink (U. S. D. A. Bul. 239, 1915) conducted a careful series of experiments in

the control of the egg-plant lace-bug, *Gargaphia solani* Heid. at Norfolk, Va., in which he carefully tested the comparative merits of soap alone and in combination with various strengths of nicotine sulfate. His results show "That the percentage of nymphs killed was but slightly affected by the increase in the amount of nicotine sulfate and the latter had no effect whatever on the adults; whereas with each increase in the amount of fish-oil soap there was a corresponding increase in the percentage of nymphs killed, until finally we arrive at a strength which will affect the adults. Above that strength we may then get perfect control of both the nymphs and adults." Fink found that eight pounds of fish oil soap in 100 gallons of water killed 100 per cent of the nymphs and 95 per cent of the adults. Wade (Okla. Agr. Exp. Sta. Bul. 116, 1917) experimenting with the control of the sycamore lace-bug, *Corythucha ciliata* Say, in Oklahoma obtained results similar to those reported by Fink and found that one pound of fish oil soap in 6 gallons of water gave the most satisfactory kill.

The writers have obtained 100 per cent kill of the nymphs and 95 per cent of the adults by using Octagon soap at the rate of one pound in eight gallons of water. In order to accurately measure the results the total number of nymphs and adults were counted on certain leaves on each plant and checks were left as usual. Care was taken to thoroly wet the underside of each leaf and to apply as nearly as possible a uniform amount of spray material to each plant.

It must be remembered that it is difficult to obtain quite so high a percentage of efficiency in commercial control on account of the difficulty of thoroly wetting the underside of all of the leaves where the insects are clustered. A fine driving mist under comparatively high pressure is essential and the application must be repeated as often as the increasing numbers of the bugs seem to make it necessary.

ACKNOWLEDGMENTS

The writers wish especially to express their thanks to Dr. Carl J. Drake, Head of the Department of Entomology, Iowa State College, for much assistance in supplying bibliographical and distribution data, the description of the adult insect and checking over the manuscript. Prof. C. R. Crosby of Cornell University has been good enough to send typewritten copies of several references needed. Mr. Harry G. Barber, Curator of Hemiptera, United States National

Museum was very helpful to the senior author while in the Museum in checking the identity of doubtful specimens in the collection.

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Explanation of Plates

Plate XL. Bean leaves showing characteristic yellowing and spotting due to lace bug feeding.

Plate XLI. Bean leaves showing progressive types of feeding injury starting from a healthy leaf (upper left) to a badly yellowed, spotted and dried leaf (lower right).

PLATE XL.

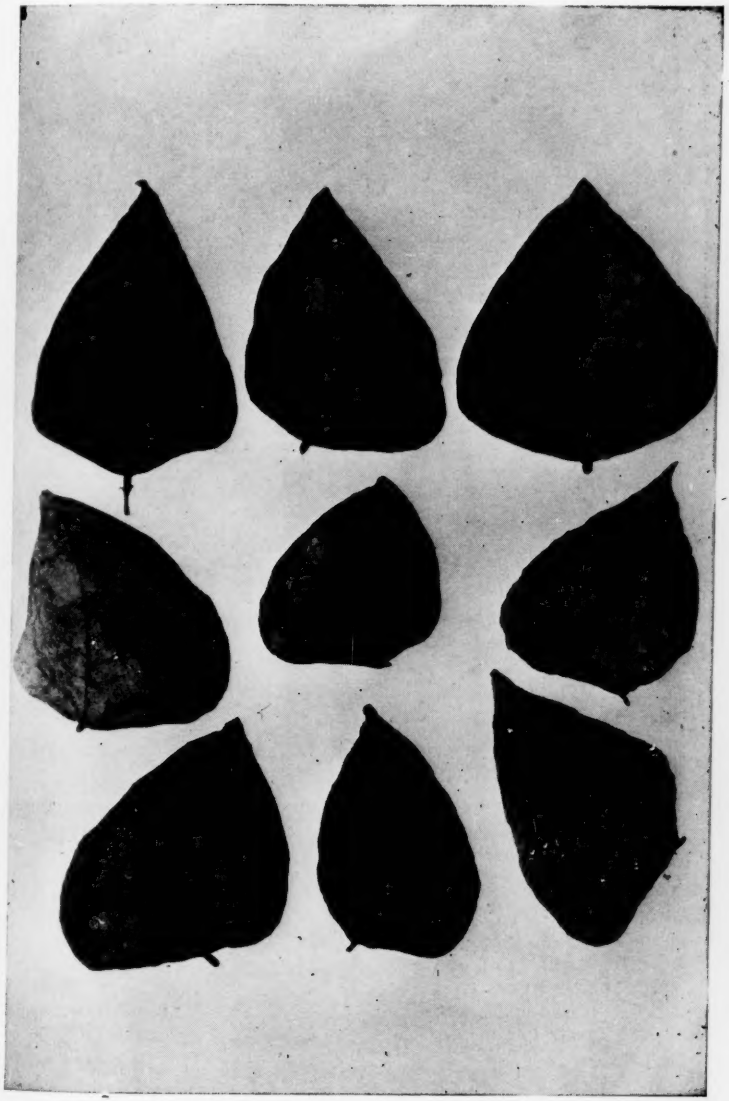
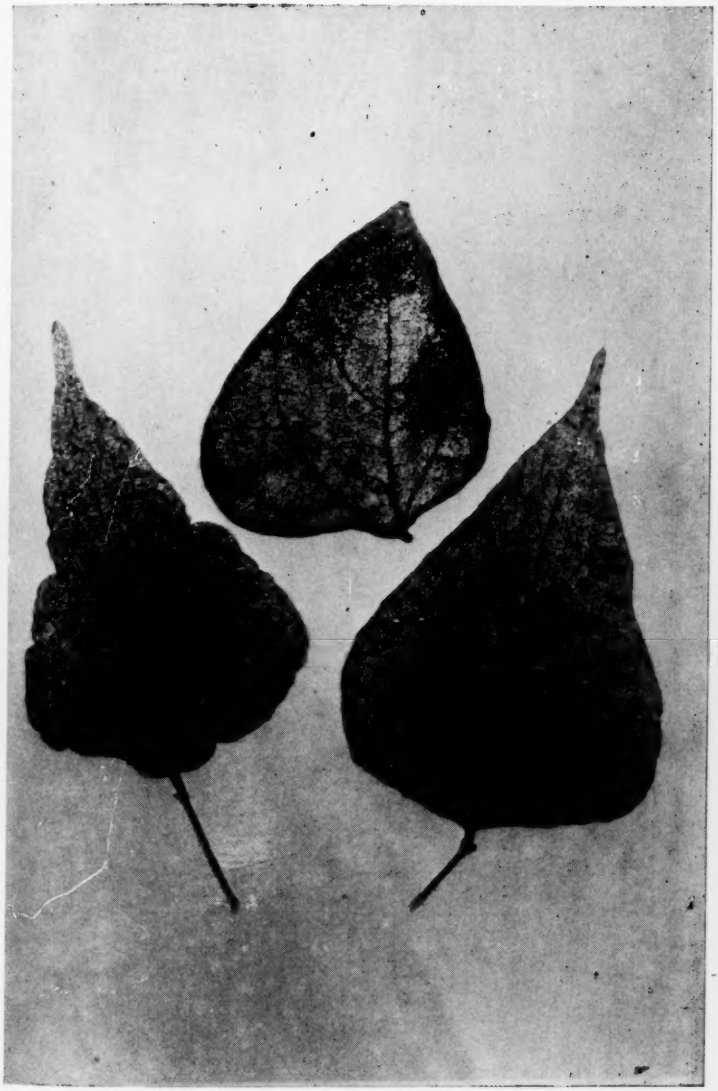


PLATE XLI.



THE HAITIAN COFFEE TREE CRICKET

By CHARLES H. ARNDT, formerly Director of the Coffee Station, and HERBERT L. DOZIER,* Entomologist, Service Technique, Port-au-Prince, Haiti

Haiti is very fortunate in that many serious coffee diseases and insect pests are not found in that country. This is somewhat offset, however, by the damage caused by a native tree cricket, recently described as *Chremon repentinus* Rehn.[1] This species in its sum total of features is probably nearest *Stenogryllus* Saussure, a genus also known only from Santo Domingo. It is related to some African forms and it was at first thought that the species might possibly have come to Haiti in early slave ships.

DISTRIBUTION

This cricket was not recognized as a serious pest until recent years when the damage it caused to young coffee plantations was observed. It is assumed that this insect is confined to the island of Hispaniola as it has not been reported to date from any other country where coffee insects have been seriously studied. It is widely distributed in Haiti and its work has been observed in the Massif de la Hotte, Rochelois Plateau, Massif de la Selle and the Massif du Nord, from sea level to an altitude of 4,000 feet.

ECONOMIC IMPORTANCE AND HOST PLANTS

It may possibly be a misnomer to call this insect "The coffee tree cricket" because it is indigenous to the island while coffee is an introduced plant. It has found coffee a very suitable plant for egg deposition and probably causes more damage to it than to any other plants of economic importance, thereby justifying the popular name assigned to it.

In the Fond-des-Negres valley this cricket lays numerous eggs in the stems of cotton but the damage does not seem to be serious on this host except in some instances where the stem becomes infected by parasitic fungi through the egg punctures. Other plants in which eggs are laid but on which the damage is of relatively little economic importance are Spanish cedar, *Cedrella odorata;* "Bois chene", *Catalpa longissima* (frequent); "Bois d'Orme", *Guazuma ulmifolia* (occasional); "Bois crapaud", *Psychotria*

[1] A New Genus of Eneopterinae from Hispaniola, Trans. Amer. Ent. Soc., 50: 87–92, 1930.

* Co-authors.

Brownei (frequent); castor oil, *Ricinus communis* (rare); mahogany, *Swietenia mahogani* (frequent); *Piper* spp. (occasional); *Russelia equisetiformis* (frequent); petioles of "Pistache des Indes", *Stercula apetela;* sour orange, avocado, saman, and cacao (rare). The terms in brackets indicate the relative frequency with which the egg punctures are found on the various plants as observed at the Fond-des-Negres coffee station. Native plants in which an abun-

FIG. 1.—Wilting and Death of the Top of a Young Coffee Tree Following Egg Punctures by the Coffee Tree Cricket, and Subsequent Infection of the Stem by the *Fusarium* Fungus; Note Accessory Up-right Shoots Which Developed After the Injury.

dance of eggs can usually be found are "liane barrique", *Trichostigma octandra,* and two species of *Hamelia,* one of which is the common "bois corail" of the gardens, *Hamelia erecta.* Eggs have also been found in the stems of two native weeds, one belonging to the Menthaceae, the other to the Compositae. All plants of the latter two groups listed are mostly weeds which grow along water courses. Emphasis should therefore be placed on keeping all areas around the plantation free of these weeds.

NATURE OF INJURY

These crickets are very inconspicuous, hiding during the day beneath rubbish, old leaves, etc., and feeding at night. During the egg-laying period the females may be readily found at night by

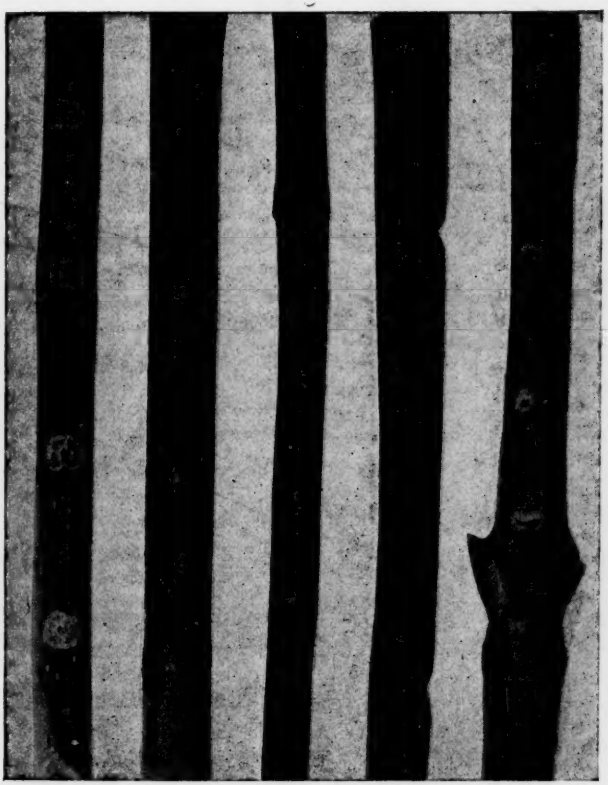


FIG. 2.—Stems of "Liane Barrique", *Trichostigma octandra*, Cotton and Coffee Showing Egg Punctures of *Chremon repentinus* Rehn, Natural Size.

searching among the coffee trees with a flash-light. They are then easily captured while in the act of oviposition. The males are very rarely found and are much less abundant than the females.

One's attention is usually first attracted to the injury by this

insect by what appear to be holes in the recent growth of the upright stems. These "holes" are made by the removal of the bark from the wood over a somewhat rounded area of about 5 mm. in diameter (Fig. 2). In the center of this area will be seen a small hole which extends into the pith. This hole is made mostly by the ovipositor for the insertion of the eggs into the pith of the stem. From three to six yellowish eggs are laid lengthwise in the pith above and below the hole. Stems from 5–9 mm. in diameter which

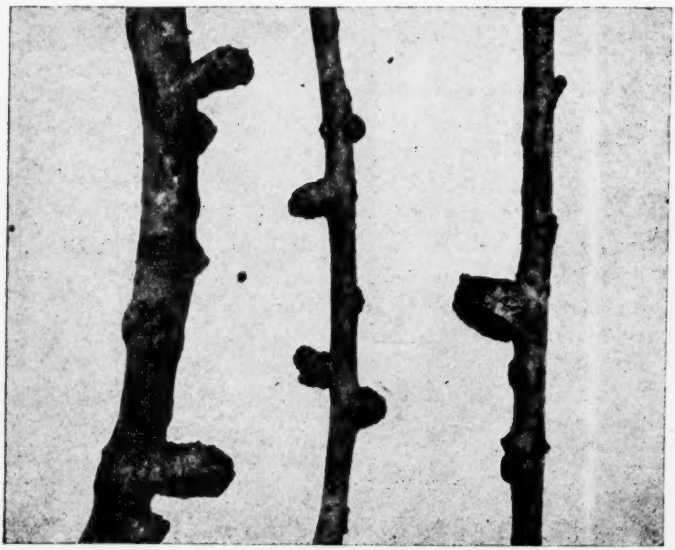


FIG. 3.—Hypertrophy of Stem of Haitian Oak, *Catalpa longissima*, a Condition Which Frequently Occurs After Oviposition Punctures by *Chremon repentinus*, Reduced.

are still green, with the wood soft, and having a large amount of pith seem to be preferred. Holes are rarely made in stems in which the wood has become well hardened. For this reason few punctures are made on the laterals because their wood becomes hardened before they are of a sufficient diameter to interest the crickets for oviposition.

The removal of the bark by the cricket over a larger area than is necessary for the insertion of the ovipositor for egg-laying, seems

to be a habit adaptation to prevent the plant from closing the hole rapidly by a callus formation and thus smothering the developing eggs. The holes are, nevertheless, frequently closed by a callus when they are made on rapidly enlarging stems. This frequently occurs on the Haitian oak where very noticeable hypertrophies as large as 1×1.5 cm. may be formed (Fig. 3).

The egg punctures themselves would injure the coffee plants little if it were not for secondary effects. The first of these is the tendency of the punctures to weaken the stems mechanically so that they are likely to break later when the laterals above the punctures set a heavy crop of berries. This weakening effect would be much less and of relatively little importance if it were not for the frequent infection of the xylem through these holes by parasitic fungi. A species of Fusarium * (Fig. 4) is the organism most commonly attacking the wood through these punctures, causing a discoloration of the vascular cylinder, but occasionally *Cercospora coffeicola* is the infecting fungus.

The *Fusarium* first attacks the pith and then grows into the wood and bark. White masses of fruiting mycelium appear on the surface of the stem adjacent to the infected puncture. The bark and pith become dark in color and the cricket eggs are frequently destroyed. It is only rarely that an old cricket hole is found which is not infected by some fungus. Frequently the infection does not occur until after the eggs have hatched, or if infection has occurred earlier the growth of the mycelium will not be sufficient to destroy the eggs. When young stems are infected, the stem above the point of attack is frequently killed. The leaves of the top will suddenly wilt, then become brown, and later brownish-black before falling. In certain plots at the Coffee Station at Fond-des-Negres as many as 60 per cent of the tops of the coffee trees were killed by such infections which followed cricket punctures. The egg punctures are usually from 2 to 3 cm. apart although occasionally they may be placed closer and at times the punctures may be opposite each other. As many as fifty-two punctures have been counted on a 60 cm. length of a coffee stem. The largest number of punctures observed for a single stem was 218 in a 340 cm. portion of a *Trichostigma octandra* stem.

* Provisionally determined by Dr. C. D. Sherbakoff, as very similar to if not identical with *Fusarium martii* A. & W.

The most serious effect of the cricket injury to the stem lies in the effect of any injury to the upper portion of the coffee tree upon its configuration or shape. Such injuries to the upright stem remove

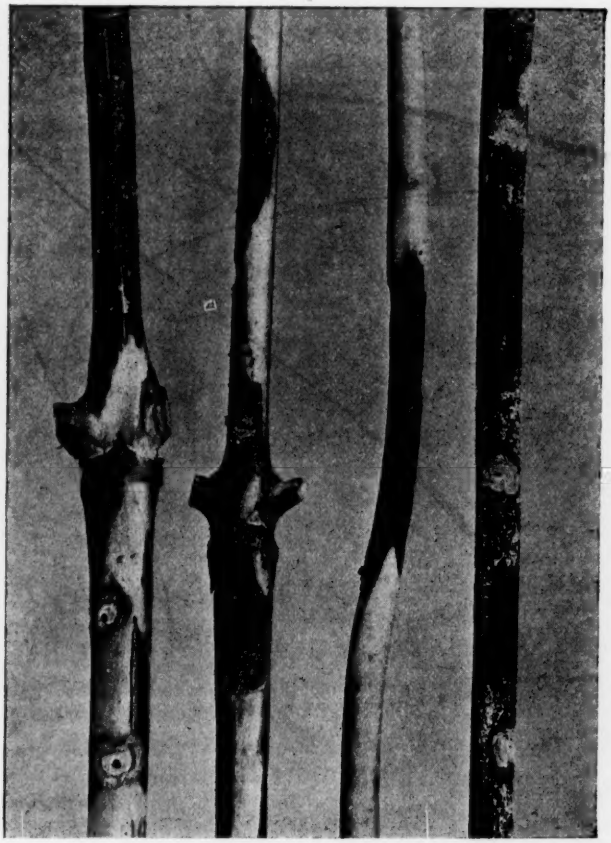


FIG. 4.—Coffee Stems Showing Various Degrees of Infection by *Fusarium* Disease, Following Egg Punctures, Natural Size.

the inhibition of the terminal bud on the normally dormant secondary buds. The removal of this inhibition results in the development of an entanglement of uprights and laterals which is very

difficult to control. When the trees are regularly damaged each year by injuries of this nature it is impossible to follow any particular system of pruning. The pruning will be difficult and will have to be directed toward the utilization of the less-injured and the uninjured shoots for the development of the fruiting branches. There is no question of the seriousness of the damage which may be caused by this cricket and unless some means is discovered to reduce its injury below that which has been observed at the Coffee Experiment Station, it will remain a serious handicap to commercial coffee growing on the Island. The older and less rapidly growing trees are less seriously injured because of the relatively lesser amount of stem in which eggs may be laid. Several observations indicate that this cricket causes more damage to coffee in sunny places than to plants located under shady conditions.

SEASONAL HISTORY

Egg-laying occurs most commonly after the rapid growth of the shoots begins in the spring, although it may occur at any time of the year when suitable young shoots are present. Eggs hatch from five to twelve months after deposition. All eggs of the same puncture hatch at about the same time, although eggs laid in the same stem but at different punctures may hatch at different times. Eggs which are laid in April, May, and June, will usually begin to hatch the following December. Most of them, however, will not hatch until several months later. The adults and nymphs are difficult to find but the very young ant-like nymphs may be found at almost any time of the year by searching carefully among the dense coffee foliage. They are most abundant from March to June.

The nymphs and adults seem to feed mostly at night and their food apparently consists mainly of scale insects, plant lice and other small insects. In our rearing cages they were constantly supplied with fresh *Saisettia hemispherica* and *Coccus viridis*, two scales commonly present on coffee foliage and stems. They appear to be distinctly cannibalistic when confined with each other in close quarters. One batch of nymphs hatched May 10th yielded a single male survivor which reached maturity August 10th, 1930. Other partial rearing records coupled with field observations show that the time from hatching to adult requires from eighty to ninety days.

DESCRIPTION OF STAGES

Egg (Fig. 5.)—Whitish in color, semi-translucent, elongate cylindrical. Without a distinct cap area differentiated, as is commonly found with many species of tree crickets. The head of the developing embryo is always oriented toward the opening. Average length 3.25 mm.; greatest width .788 mm.

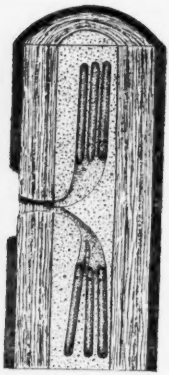


FIG. 5.—Diagramatic longitudinal section of stem to show manner of oviposition.

Nymph.—First instar: Color reddish-brown, the abdomen darker; hind femora banded with white; basal third of antennae pale, remainder dark; cerci pale. Resembles a small ant. Length 3.5-5.2 mm.

Second instar: General color brownish-black, the base of abdomen and sides of thorax marked with yellowish-orange; a very narrow cross-band on abdomen pale; antennae black except pale base; cerci black. Length 5.2–8.3 mm.

Third instar: General color brownish-black, the thorax for the most part tawny, abdomen with narrow pale cross band; legs black except longitudinal pale markings and distinctly paler base of femora; antennae black, pale at base; cerci blackish at tips, lightening towards base. Length 8.3–11.4 mm.

Fourth instar: Color much lighter than in preceding stage; head except front part, thorax and legs, pale yellowish-testaceous, the thorax outlined with black borders, and the hind femora with several longitudinal black markings; antennae black, banded with yellow at base and about the middle; cerci pale, black at tip. Length 11.4–15.5 mm.

Fifth instar: Distinctly paler, the head pale except the eyes and front part, dark; thorax outlined with narrow black border; abdomen for the most part dark, central median portion of each segment pale; wing pads pale, the venation and outer sides somewhat darker; legs pale, with lineate longitudinal dark streaking on femora. Antennae yellow except a very narrow black band a short distance from the base; cerci pale; ovipositor pale. Length 15.5-22. mm.

Adult (Fig. 6)—General color of head, pronotum and legs pale ochraceous-orange; abdomen brown with pale ochraceous-orange evident on dorsal surface; fore part of head and eyes fuscous-

brown; antennae pale yellowish-orange; pronotum with the entire border margined with brownish-black; tegmina or wing covers a pale clay color, the veins dull yellow. Legs pale, ventral margins of the femora and dorsal surface of the middle and hind femora lineate with fuscous-brown. Ovipositor brownish-black. The sexes may be at once distinguished by the difference in shape and vena-

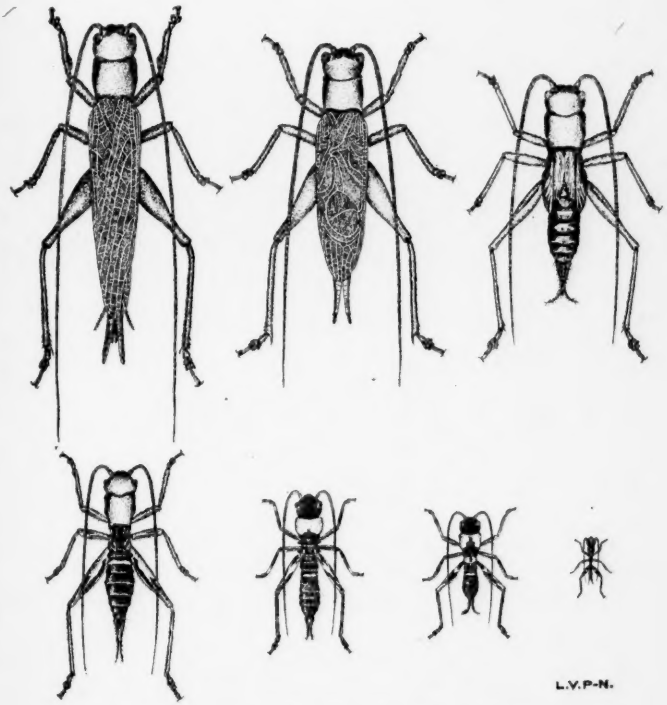


FIG. 6.—Adult Female and Male and Stages of Development of the Coffee Tree Cricket, Enlarged One-third.

tion of the tegmina. In the female the tegmina are very elongate, distinctly longer and more slender than with the male, the apices surpassing the tips of the tegmina. In the male the tegmina are broader with very different venation and with a sub-ovate tympanum. Length of male, 18.9–22. mm.; length of female, including ovipositor, 26 mm.

SAN JUAN, P. R.
BUREAU OF SUPPLIES, PRINTING, AND TRANSPORTATION
1931